



**HAL**  
open science

## A Soluble Metabolon Synthesizes the Isoprenoid Lipid Ubiquinone

Mahmoud Hajj Chehade, Ludovic Pelosi, Cameron David Fyfe, Laurent Loiseau, Bérengère Rascalou, Sabine Brugière, Katayoun Kazemzadeh, Chau-Duy-Tam Vo, Lidia Ciccone, Laurent Aussel, et al.

► **To cite this version:**

Mahmoud Hajj Chehade, Ludovic Pelosi, Cameron David Fyfe, Laurent Loiseau, Bérengère Rascalou, et al.. A Soluble Metabolon Synthesizes the Isoprenoid Lipid Ubiquinone. *Cell Chemical Biology*, 2019, 26 (4), pp.482-492.e7. 10.1016/j.chembiol.2018.12.001 . hal-02071798

**HAL Id: hal-02071798**

**<https://hal.science/hal-02071798>**

Submitted on 12 Nov 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1           **A soluble metabolon synthesizes the isoprenoid lipid Ubiquinone**

2  
3 Mahmoud Hajj Chehade<sup>1</sup>, Ludovic Pelosi<sup>1</sup>, Cameron David Fyfe<sup>2</sup>, Laurent Loiseau<sup>3</sup>, Bérengère  
4 Rascalou<sup>1</sup>, Sabine Brugière<sup>4</sup>, Katayoun Kazemzadeh<sup>1</sup>, Chau-Duy-Tam Vo<sup>2</sup>, Lidia Ciccone<sup>5</sup>, Laurent  
5 Ausseil<sup>3</sup>, Yohann Couté<sup>4</sup>, Marc Fontecave<sup>2</sup>, Frédéric Barras<sup>3,6</sup>, Murielle Lombard<sup>2</sup>, Fabien Pierrel<sup>1\*</sup>

6  
7 <sup>1</sup> Univ. Grenoble Alpes, CNRS, Grenoble INP, TIMC-IMAG, F-38000 Grenoble, France

8 <sup>2</sup> Laboratoire de Chimie des Processus Biologiques, Collège de France, Université Pierre et Marie Curie,  
9 CNRS UMR 8229, PSL Research University, 11 place Marcelin Berthelot, 75005 Paris, France.

10 <sup>3</sup> Aix Marseille Université, CNRS, Laboratoire Chimie Bactérienne, Institut Microbiologie de la  
11 Méditerranée, 31 Chemin Joseph Aiguier, Marseille 13009, France

12 <sup>4</sup> Univ. Grenoble Alpes, CEA, Inserm, BIG-BGE, 38000 Grenoble, France

13 <sup>5</sup> SOLEIL Synchrotron, L'Orme des Merisiers, 91198 Gif-sur-Yvette, France

14 <sup>6</sup> SAME Unit, Department de Microbiologie, Institut Pasteur, 25 Rue du Dr Roux, 75015 Paris, France

15 \* Correspondence: [fabien.pierrel@univ-grenoble-alpes.fr](mailto:fabien.pierrel@univ-grenoble-alpes.fr)

16 Lead contact: Fabien Pierrel

17  
18 **Key words:** metabolon, multiprotein complex, lipid biosynthesis, ubiquinone, SCP2, metabolic  
19 pathway

20

21 **ABSTRACT:**

22           Ubiquinone (UQ) is a polyprenylated lipid that is conserved from bacteria to humans and is  
23 crucial to cellular respiration. How the cell orchestrates the efficient synthesis of UQ, which involves  
24 the modification of extremely hydrophobic substrates by multiple sequential enzymes, remains an  
25 unresolved issue. Here, we demonstrate that seven Ubi proteins form the Ubi complex, a stable  
26 metabolon that catalyzes the last six reactions of the UQ biosynthetic pathway in *Escherichia coli*. The  
27 SCP2 domain of UbiJ forms an extended hydrophobic cavity that binds UQ intermediates inside the 1  
28 MDa Ubi complex. We purify the Ubi complex from cytoplasmic extracts and demonstrate that UQ  
29 biosynthesis occurs in this fraction, challenging the current thinking of a membrane-associated  
30 biosynthetic process. Collectively, our results document a rare case of stable metabolon and highlight  
31 how the supramolecular organization of soluble enzymes allows the modification of hydrophobic  
32 substrates in a hydrophilic environment.

### 33 INTRODUCTION:

34 Isoprenoid quinones are widespread in the three domains of life and represent a peculiar  
35 group of lipids able to undergo a two-step reversible redox process (Nowicka and Kruk, 2010). The  
36 electron transfer properties of isoprenoid quinones are essential to the function of respiratory chains  
37 that generate cellular ATP (Schoepp-Cothenet et al., 2013). Isoprenoid quinones also mediate disulfide  
38 bond formation in proteins (Bader et al., 2000), participate to virulence and antibiotic resistance in  
39 bacteria (Aussel et al., 2014b) and function as membrane soluble antioxidants (Bentinger et al., 2010;  
40 Nowicka and Kruk, 2010). The main isoprenoid quinones are menaquinone (MK) and ubiquinone (UQ),  
41 which is also called coenzyme Q in eukaryotes. MK and UQ share the same polyisoprenoid tail but  
42 differ by the structure of the redox-active head group, naphthalene ring and benzene ring, respectively  
43 (Figure S1A). The polyisoprenoid tail varies in length between organisms (UQ<sub>6</sub> in *Saccharomyces*  
44 *cerevisiae*, UQ<sub>8</sub> in *Escherichia coli* and UQ<sub>10</sub> in humans) and confers extremely hydrophobic properties  
45 to isoprenoid quinones, which localize consequently in cellular membranes (Kawamukai, 2018;  
46 Nowicka and Kruk, 2010).

47 In the UQ biosynthesis pathway, the polyisoprenoid tail is added at an early step by an integral  
48 membrane polyprenyltransferase called UbiA in *E. coli* (Figure 1A). Subsequently, seven consecutive  
49 chemical reactions modify the head group to yield UQ (Figure 1A). Hydrophobic polyprenylated  
50 intermediates of the UQ biosynthetic pathway are predicted to be embedded within the lipid bilayer  
51 (Stefely and Pagliarini, 2017), with the head groups near the glycerol backbone of the membrane  
52 phospholipids and the isoprenoid tail inserted between their acyl chains (Galassi and Arantes, 2015;  
53 Hoyo et al., 2017; Wollstein et al., 2015). In contrast, the decarboxylase, methyltransferases and  
54 hydroxylases of the UQ biosynthetic pathway contain no transmembrane domains and are predicted  
55 to be soluble. Thus, how UQ biosynthetic enzymes overcome the biophysical barrier that separates  
56 them from their lipophilic substrates constitutes an unresolved issue.

57 Besides the Ubi enzymes that catalyze the chemical reactions of the pathway (Figure 1A), three  
58 Ubi proteins of unknown function are also required for efficient UQ biosynthesis, UbiB (Poon et al.,  
59 2000), UbiJ (Aussel et al., 2014a) and UbiK (Loiseau et al., 2017). Lately, we demonstrated that purified  
60 UbiJ and UbiK form a 1:2 heterocomplex that binds palmitoleic acid, one of *E. coli* most abundant fatty  
61 acid (Loiseau et al., 2017). This capacity to bind lipids suggested that UbiJ and UbiK may act as accessory  
62 factors of Ubi enzymes, but the role of these two proteins and of UbiB, although central for UQ  
63 biosynthesis, is still undefined (Bayly and Yadav, 2017; Loiseau et al., 2017; Reidenbach et al., 2017).

64 Together, UQ biosynthesis raises generic questions about how enzymes deal with extremely  
65 hydrophobic substrates and how cells orchestrate multi-step biosynthetic pathways. In order to  
66 address these fundamental questions, we have studied the subcellular localization and the structural  
67 organization of the UQ pathway in the bacterium *E. coli*. Here, we show that five Ubi enzymes and the  
68 accessory factors UbiJ-UbiK form a ~1000 kDa Ubi complex that resides in the cytosolic fraction. We  
69 demonstrate that UbiJ binds hydrophobic UQ biosynthetic intermediates and is also the keystone of  
70 the Ubi complex. Our work describes the Ubi complex as a unique microreactor, which enables the  
71 multistep biosynthesis of a complex lipid in a hydrophilic cellular environment.

72

## 73 **RESULTS:**

### 74 ***UQ biosynthesis takes place in the soluble fraction of E. coli lysates***

75 An assay to monitor UQ biosynthesis *in vitro* is not available (Stefely and Pagliarini, 2017). We  
76 designed an *in vivo* experimental approach to accumulate a labelled version of OPP within cells and to  
77 monitor its conversion into labelled UQ. We transferred  $\Delta ubiC$  cells pretreated with chloramphenicol  
78 in an O<sub>2</sub>-free atmosphere and added the labelled precursor <sup>13</sup>C<sub>7</sub>-4HB. The absence of dioxygen blocked  
79 UQ biosynthesis (Shestopalov et al., 1997), and the cells quickly accumulated <sup>13</sup>C<sub>6</sub>-OPP (Figure S1B).  
80 Upon exposure to air, cells preloaded anaerobically with <sup>13</sup>C<sub>6</sub>-OPP synthesized <sup>13</sup>C<sub>6</sub>-UQ<sub>8</sub> (Figure S1C).

81 Conversely, we observed no  $^{13}\text{C}_6\text{-UQ}_8$  synthesis in cells maintained under argon atmosphere (Figure  
82 S1C). Thus, dioxygen triggered the *in vivo* conversion of  $^{13}\text{C}_6\text{-OPP}$  into  $^{13}\text{C}_6\text{-UQ}_8$  in our experimental set-  
83 up, in agreement with the hydroxylases UbiF, H and UbiI requiring  $\text{O}_2$  as a cosubstrate (Alexander and  
84 Young, 1978; Hajj Chehade et al., 2013) (Figure 1A).

85 Next, we lysed cells loaded with  $^{13}\text{C}_6\text{-OPP}$  and found that total cell free extracts incubated  
86 under air at  $30^\circ\text{C}$  also synthesized  $^{13}\text{C}_6\text{-UQ}_8$  (Figure S1D). The biosynthesis of  $\text{UQ}_8$  was strongly  
87 stimulated by the addition of S-adenosylmethionine and NADH (Figure S1D), which act respectively as  
88 the methyl-donor for the methylation reactions and as the electron-donor for the hydroxylases (Knoell,  
89 1979b). Finally, we fractionated the cell free extracts by ultracentrifugation and assayed the  
90 cytoplasmic and membrane fractions for UQ biosynthesis. Only the cytoplasmic fraction produced  $^{13}\text{C}_6\text{-}$   
91  $\text{UQ}_8$  upon exposure to air (Figure 1B and S1E). The initial rates were comparable between the soluble  
92 fraction and cell free extracts (Figure 1B). However increased  $^{13}\text{C}_6\text{-UQ}_8$  synthesis was observed at later  
93 stages in cell free extracts (Figure 1B), potentially reflecting limiting amounts of the  $^{13}\text{C}_6\text{-OPP}$  substrate  
94 in the soluble fraction. In conclusion, we developed an activity assay, which strongly supports that the  
95 last six reactions of the UQ biosynthetic pathway take place in the cytosol.

#### 96 ***Ubi proteins are found predominantly in the soluble fraction of E. coli lysates***

97 The presence of UQ biosynthesis activity in the cytoplasmic fraction prompted us to evaluate  
98 the distribution of Ubi proteins. We created several Ubi-SPA strains by inserting a sequence coding for  
99 the SPA tag at the 3' end of *ubi* genes in the *E. coli* chromosome (Figure S1F). The tagged proteins were  
100 functional since the  $\text{UQ}_8$  content of the strains accounted for at least 70% of WT level (Figure S1G).  
101 Western blot analysis of cellular extracts with an anti-Flag antibody detected the Ubi proteins at their  
102 expected size, except the membrane-bound UbiA and UbiB that did not show any signal (data not  
103 shown). Ultracentrifugation of cell free extracts separated soluble and membrane fractions as verified  
104 by immunodetection of the cytosolic glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the  
105 membrane-bound porin LamB (Figure 1C). All tested Ubi-SPA proteins distributed in both fractions

106 (Figure 1C). We calculated the total amount of each Ubi protein in the entire soluble and membrane  
107 fractions. The ratio of these amounts indicate the distribution of each Ubi protein (Figure 1C) and  
108 showed that all Ubi proteins localized predominantly in the soluble fraction, except UbiK.

#### 109 ***Seven Ubi proteins form a 1 MDa complex***

110 To further characterize the soluble Ubi proteins, we analyzed cytoplasmic fractions by Blue  
111 Native PAGE (BN-PAGE), a technique that preserves the integrity of protein complexes and separates  
112 them according to their hydrodynamic size and shape (Wittig et al., 2006). Anti-Flag immunodetection  
113 showed that seven Ubi-SPA proteins (UbiE, F, G, H, I, J, K) co-migrated at ~1000 kDa, whereas UbiD and  
114 UbiX co-migrated at around 700 kDa (Figure 2A). Similar results were obtained with an anti-CBP  
115 antibody, demonstrating the specific detection of the SPA tag fused to the Ubi proteins (Figure S2A).  
116 As expected, the tetrameric GAPDH protein (4x35.5 kDa) ran at ~140 kDa (Figure 2A and S2A). We also  
117 subjected the samples to a denaturing second dimension electrophoresis (2D SDS-PAGE) after the  
118 first dimension BN-PAGE (1D BN-PAGE). 2D SDS-PAGE confirmed the migration of UbiE, F, G, H, I, J, K  
119 proteins at ~1000 kDa and of UbiX and UbiD at ~700 kDa (Figure 2B). GAPDH was detected at ~100  
120 kDa with variations between experiments due to migration artefacts in the lower part of the gel and  
121 to disparities in the positioning of the 1D BN-PAGE lane (Figure S2B).

122 To corroborate the co-migration of several Ubi proteins, we used a second method that  
123 separates proteins in glycerol gradients according to their density. UbiE, F, G, H, I, J, K distributed  
124 around the high density fractions 11 to 16, whereas UbiD and X were most abundant in fractions 9 to  
125 13, and GAPDH eluted in fractions 6 to 10 (Figure 2C). Silver staining revealed that most proteins of  
126 the soluble extracts localized in low-density fractions (Figure 2C). We analyzed by 2D SDS-PAGE  
127 fractions 11, 12 and 13 from the glycerol gradient of UbiG-SPA and obtained a signal for the UbiG  
128 protein at ~1000 kDa (Figure 2D). Thus, both BN-PAGE and glycerol gradient experiments consistently  
129 established the existence of the same protein complex that we name “Ubi complex”.

130 To provide additional proof of the existence of the Ubi complex, we performed bacterial two-  
131 hybrid experiments, in which adenylate cyclase (AC) activity reports on the interaction between Ubi  
132 proteins fused either to the T18 or T25 domains of AC (Karimova et al., 1998). We tested all  
133 combinations (Table S1) and obtained an interaction network where the seven proteins of the Ubi  
134 complex exhibited multiple robust interactions, whereas the other Ubi proteins were less connected  
135 (Figure 2E). Collectively, our results are consistent with UbiE, F, G, H, I, J, K being part of a cytoplasmic  
136 1 MDa Ubi complex, whereas UbiX and UbiD involved in the decarboxylation step form a separate  
137 entity.

#### 138 ***Purification of the Ubi complex and identification of its components***

139 Next, we isolated the Ubi complex using sequential affinity purification, a two-step procedure  
140 that uses first the FLAG epitopes of the SPA tag and second its CBP domain (Babu et al., 2009). We  
141 processed soluble extracts from UbiE-SPA cells and from WT untagged cells as control. Both eluates  
142 from the anti-FLAG resin were similar (Figure S2C-D) but immunodetection confirmed the presence of  
143 UbiE-CBP in the UbiE-SPA sample (Figure S2E). E2-E4 eluates of the calmodulin resin showed multiple  
144 bands that were absent in the control eluate (Figure S2F-G) and contained the bait UbiE-CBP protein  
145 as revealed by western blot analysis (Figure S2H). We detected the purified Ubi complex at ~1000 kDa  
146 in a 2D SDS-PAGE analysis of the E2 eluate (Figure 3A), with a signal similar to the Ubi complex in total  
147 soluble fractions (Figure 2B). We similarly purified the Ubi complex from UbiI-SPA and UbiG-SPA strains  
148 and obtained E2 fractions whose SDS-PAGE shared protein bands with the UbiE-SPA fraction E2 (Figure  
149 3B). To identify the proteins in the Ubi complex, all three samples were submitted to mass  
150 spectrometry (MS)-based proteomic analysis and compared with E2 fractions from independent  
151 control purifications. The stringent comparison of MS-based results obtained for SPA-tagged proteins  
152 and negative controls identified several proteins that co-purified with the bait proteins (Table S2).  
153 Interestingly we detected only seven Ubi proteins and all of them were found in a subset of 8 proteins  
154 systematically enriched in the three Ubi-SPA eluates (Figure 3C, Table S2). The 8 proteins were UbiE,



155 F, G, H, I, J, K and the lipoprotein Blc, which physiological function remains uncertain (Campanacci et  
156 al., 2006). The UQ<sub>8</sub> content of the  $\Delta blc$  strain was comparable to WT (Figure S2I), showing that the Blc  
157 protein is not required for UQ biosynthesis, unlike Ubi proteins of the Ubi complex. Overall, we  
158 conclude that the biosynthesis of UQ in the soluble fraction (Figure 1B) is mediated by the Ubi complex,  
159 which contains the five enzymes (UbiE, F, G, H, I) necessary to convert OPP to UQ<sub>8</sub> (Figure 1A) and two  
160 accessory factors, UbiJ and UbiK.

#### 161 ***UQ biosynthetic intermediates OPP and DMQ<sub>8</sub> co-elute with the Ubi complex***

162 Using HPLC coupled to electrochemical detection and mass spectrometry (HPLC-ECD-MS), we  
163 analyzed the distribution of isoprenoid quinones in exponentially growing *E. coli* cells and found that  
164 the UQ intermediates OPP and DMQ<sub>8</sub> accumulated substantially in the cytosolic fraction, whereas UQ<sub>8</sub>  
165 was highly enriched in the membrane fraction (Figure 4A). Analysis of the cytosols of Ubi-SPA strains  
166 on glycerol gradients revealed that OPP and DMQ<sub>8</sub> were enriched in fractions that contain the Ubi  
167 complex, as visualized by western-blot analysis (Figure 4B and S3A-B). Conversely, UQ<sub>8</sub> distributed  
168 quite evenly in the gradient (Figure 4B and S3A-B). Together, these results showed that the  
169 hydrophobic intermediates OPP and DMQ<sub>8</sub> localized to a significant extent in the soluble fraction of *E.*  
170 *coli* extracts and co-eluted with the Ubi complex.

#### 171 ***The SCP2 domain of UbiJ binds isoprenoid lipids***

172 We recently showed that the purified UbiJ-UbiK heterocomplex binds palmitoleic acid (Loiseau  
173 et al., 2017), one of the most abundant fatty acid in *E. coli*. Overexpression of UbiJ-K caused a decrease  
174 in cellular UQ<sub>8</sub> levels and the accumulation of OPP and DMQ<sub>8</sub> (Figure 4C and S3C), suggesting the  
175 sequestration of these UQ<sub>8</sub> biosynthetic intermediates by UbiJ-K. We then purified the UbiJ-K complex  
176 (Figure S3D) using affinity chromatography and size-exclusion chromatography, and analyzed its  
177 quinone content by HPLC-ECD-MS (Figure S3E). We detected UQ<sub>8</sub>, OPP and DMQ<sub>8</sub> (Figure 4D), showing  
178 that UbiJ-K is able to bind all three isoprenoid lipids. To elucidate which of the two proteins is  
179 responsible for lipid binding, we purified UbiK and the N-terminal SCP2 domain of UbiJ (UbiJ-SCP2)

180 (Figure S5E); full-length UbiJ was not amenable to purification. We detected UQ<sub>8</sub>, OPP and DMQ<sub>8</sub> in  
181 UbiJ-SCP2, but not in UbiK samples (Figure 4D and S3E). The calculated UQ<sub>8</sub> /protein ratio showed a  
182 low occupancy in UbiJ-K and UbiJ-SCP2 (less than 0.3 %), probably due to the high yield expression and  
183 the multiple purification steps. Together, our results strongly support that the SCP2 domain of UbiJ,  
184 and not UbiK, is responsible for binding UQ<sub>8</sub> and the biosynthetic intermediates OPP and DMQ<sub>8</sub> in the  
185 Ubi complex.

### 186 ***Structure of the SCP2 domain of UbiJ***

187 UbiJ-SCP2 behaved as a dimer in solution and crystallized in the space group P212121 with an  
188 asymmetric unit containing either a dimer or a tetramer (Table S3). The structure shown in Figure 5A  
189 is that of the dimer generated with data collected to the highest 1.7 Å resolution. An electron density  
190 map is shown in Figure S4. The protein has an  $\alpha/\beta$ -fold with 5  $\alpha$ -helices and 5  $\beta$ -strands forming a  
191 hydrophobic center with an open cavity (Figure 5B). The walls of this cavity are composed of  
192 hydrophobic residues provided both by the  $\alpha$ -helices and the  $\beta$ -strands (Figure 5C-D). It is interesting  
193 to note that, at those specific positions, UbiJ-SCP2 domains have exclusively hydrophobic residues  
194 (Figure S5). The overall structure, as well as the presence of a hydrophobic cavity, is highly reminiscent  
195 of previously reported structures of the SCP2 protein family (Table S4). This suggests that the absence  
196 of the C-terminal part of UbiJ does not perturb the SCP2 domain in line with our model showing two  
197 independent domains (Loiseau et al., 2017). In some cases, SCP2 proteins have been crystallized in  
198 complex with a hydrophobic ligand as a further evidence for the cavity playing a functional role in lipid  
199 binding (Table S4) (Burgardt et al., 2017; De Berti et al., 2013; Dyer et al., 2008; Haapalainen et al.,  
200 2001). Although the model we present does not contain any ligand, electron density was noted within  
201 the cavity (Figure 5C). It is tempting to suggest that this unexplained density is related to the various  
202 lipids detected in small quantities within the purified protein (see above) and that the cavity of UbiJ  
203 serves as the binding site for UQ<sub>8</sub> intermediates. As a final support to that hypothesis we found that a  
204 UQ<sub>8</sub> conformation taken from a previously reported structure of photosynthetic LH1-RC protein in

205 complex with UQ<sub>8</sub> (Yu et al., 2018) nicely aligns into this cavity, with a reasonable fit in length (Figure  
206 5E).

207

### 208 ***UbiJ is essential to the stability of the Ubi complex***

209 Our characterization of UbiJ as a polyprenyl lipid-binding protein led us to characterize OPP  
210 binding to the Ubi complex in  $\Delta ubiJ$  cells.  $\Delta ubiJ$  cells are blocked at an undefined early step of the UQ  
211 biosynthetic pathway (Aussel et al., 2014a) and have therefore a strongly diminished UQ<sub>8</sub> content and  
212 increased OPP levels compared to WT cells (Figure S6A). The cytosol of UbiG-SPA  $\Delta ubiJ$  cells analyzed  
213 on glycerol gradient showed a profile with comparable amounts of OPP and UQ<sub>8</sub> in all fractions (Figure  
214 6a). A similar result was obtained with UbiF-SPA  $\Delta ubiJ$  and UbiH-SPA  $\Delta ubiJ$  cells (Figure S6B-C). This  
215 distribution of OPP is in stark contrast with the accumulation of OPP in the Ubi complex observed in  
216 cells that contain UbiJ (Figure 4B and S3A-B).

217 The deletion of *ubiJ* did not affect the steady state level of UbiF or UbiG but lowered that of  
218 UbiH (Figure S6B). Western-blot analyses revealed that the Ubi proteins shifted from fractions 11-16  
219 (Figure 2c) in WT cells to fractions 2-10 in  $\Delta ubiJ$  cells (Figure 6A and S6C-D), whereas the position of  
220 GAPDH was unchanged (compare Figure 6A and 2C). BN-PAGE and 2D-SDS-PAGE analyses showed that  
221 the Ubi complex was absent in  $\Delta ubiJ$  cells (Figure 6B-D). Conversely, the Ubi complex was readily  
222 detected in cells lacking *ubiE*, *ubiF*, *ubiG* (Figure 6B-C) or *ubiK* (Figure 6E), even though deletion of this  
223 last gene decreased UbiI levels (Figure S6E). Interestingly, cells with a Ubi complex lacking an enzymatic  
224 subunit like UbiF or UbiE produced substantial quantities of the late stage intermediates DMQ<sub>8</sub> and  
225 DDMQ<sub>8</sub>, respectively (Figure S6F). Together, our results show that UbiJ is required for the stability of  
226 the Ubi complex and explain why UQ biosynthesis cannot progress past the early intermediate OPP in  
227  $\Delta ubiJ$  cells.

228

229 **DISCUSSION:**

230 Here, we demonstrate the supramolecular organization of seven UQ biosynthesis proteins in  
231 a 1 MDa Ubi complex (Figure 2), which is remarkable for at least 2 reasons. First, it constitutes a stable  
232 metabolon, a term that designates the association of sequential enzymes acting in a metabolic  
233 pathway (Srere, 1985). Often, metabolons are highly dynamic and therefore not amenable to  
234 biochemical characterization (Laursen et al., 2016; Nguyen et al., 2014; Wu and Minter, 2015), like  
235 the purinosome. However, a few stable metabolons have been purified like the pyruvate  
236 dehydrogenase complex (Zhou et al., 2001) or a triacylglycerol biosynthetic complex in *Rhodotorula*  
237 *glutinis* (Gangar et al., 2001). We have shown that the Ubi complex contains two accessory factors  
238 (UbiJ, K) and the Ubi enzymes (UbiI, G, H, E, F) that catalyze the last six reactions of the UQ biosynthesis  
239 pathway. As such, the Ubi complex is, to our knowledge, ~~the first example of a~~ the stable metabolon  
240 ~~that coordinating coordinates the so many~~ highest number of chemical reactions. Substrate channeling  
241 between Ubi enzymes and control of undesired reactivity of UQ intermediates may be conferred by  
242 the Ubi metabolon to contribute to efficient UQ biosynthesis.

243 Second, the Ubi complex is present in the cytosolic fraction even though its polyprenylated  
244 substrates count among the most hydrophobic molecules in life (Nowicka and Kruk, 2010; Stefely and  
245 Pagliarini, 2017). As a matter of fact, we have experimentally established that the biosynthetic steps  
246 from OPP to UQ<sub>8</sub> proceed in the cytosolic fraction, independently from the membrane, as suggested  
247 by an early report (Knoell, 1979a) and supported by the following results: i) OPP and DMQ<sub>8</sub>, two UQ<sub>8</sub>  
248 biosynthetic intermediates, accumulated in the cytosolic fraction to a greater extent than the mature  
249 UQ<sub>8</sub> and co-purified with the soluble Ubi complex (Figure 4), ii) the Ubi metabolon was located in the  
250 soluble fraction, which actively converted OPP into UQ<sub>8</sub> *in vitro*, unlike the membrane fraction (Figure  
251 1B). We believe that UbiJ is key to create, in the Ubi complex, a microenvironment that isolates  
252 lipophilic molecules from the surrounding hydrophilic cytoplasm. Indeed, we demonstrated that the  
253 SCP2 domain of UbiJ binds UQ biosynthetic intermediates (Figure 4) and the crystal structure revealed

254 an elongated hydrophobic cavity, which size and shape is compatible with the binding of the polyprenyl  
255 tail of UQ intermediates (Figure 5). SCP2 domains are typically found lone (unfused) or in multidomain  
256 proteins and fulfill lipid-binding and transport functions in all domains of life (Burgardt et al., 2017).  
257 We demonstrated here ~~for the first time~~ the presence of a SCP2 protein in a multiprotein complex  
258 involved in lipid modification. Altogether, we propose a model of UQ<sub>8</sub> biosynthesis, in which the  
259 hydrophobic UQ intermediates downstream of OPP are not associated to membrane lipids but to UbiJ  
260 within the soluble Ubi metabolon (Figure 6F).

261         Since the early prenylation step by UbiA takes place in the membrane (Cheng and Li, 2014;  
262 Huang et al., 2014), our model postulates that the early octaprenyl intermediates (either OHB or OPP)  
263 move out of the membrane so that OPP can bind to UbiJ in the soluble Ubi complex. This mechanism  
264 is unusual because enzymes that synthesize lipids are often membrane-bound or membrane-  
265 associated, like the phospholipid biosynthetic pathways in *E. coli* (Lopez-Lara and Geiger, 2017).  
266 However, the extrusion of a lipid from the membrane to allow its chemical modification has a  
267 precedent, with the eukaryotic Sec14 that extracts phosphatidylinositol from the membrane and  
268 presents it to soluble hydroxylases (Bankaitis et al., 2010). In the case of UQ biosynthesis, we believe  
269 that the accessory factor UbiB may assist the extrusion of either OHB or OPP from the membrane  
270 (Figure 6F). Indeed, UbiB and its eukaryote homolog Coq8 were recently hypothesized to couple the  
271 hydrolysis of ATP to the extraction of head groups of UQ intermediates out of the membrane  
272 (Reidenbach et al., 2017; Stefely et al., 2016). Of note, UbiB contains one transmembrane domain and  
273 is not part of the purified Ubi complex (Table S2), consistent with the soluble nature of the latter.  
274 Reciprocally, how UQ<sub>8</sub> is delivered to the membrane bilayer after its synthesis by the soluble Ubi  
275 complex will be addressed in future studies. Overall, UbiK may also play a role in trafficking the  
276 polyprenyl molecules in and out of the membrane as a significant part of UbiK associates with the  
277 membrane (Figure 1C) and it was described to enhance the fusion of membranes *in vitro* (Carrica et  
278 al., 2011).

279 UbiJ and UbiK are conserved in many proteobacteria (Loiseau et al., 2017; Xia et al., 2017) and  
280 we demonstrated that both proteins are *bona fide* subunits of the Ubi complex. Besides binding UQ  
281 intermediates, we also found that UbiJ was an essential structural element of the Ubi complex, unlike  
282 other Ubi proteins (Figure 6B-E). We showed previously that the C-terminal domain of UbiJ was  
283 essential for UQ biosynthesis *in vivo* and formed an extended  $\alpha$ -helix that likely associated within a  
284 three-helix bundle in the UbiK<sub>2</sub>-UbiJ<sub>1</sub> heterocomplex (Aussel et al., 2014a; Loiseau et al., 2017). Taking  
285 all these findings into account, we propose that UbiJ is the keystone of the Ubi complex and constitutes  
286 a docking platform where Ubi enzymes assemble and access their SCP2-bound polyprenyl substrates.  
287 The total molecular weight of the eight proteins identified in the purified Ubi complex approaches 270  
288 kDa, still far from the estimated 1 MDa observed on BN-PAGE. The extra mass may correspond to  
289 multimeres of Ubi proteins in the Ubi complex, as supported by a study that found members of  
290 multiprotein complexes to be made in precise proportion to their stoichiometry, and showed that Ubi  
291 proteins have heterogeneous synthesis rates (Li et al., 2014). We also found that several Ubi proteins  
292 interacted with themselves in our two-hybrid assay (Table S1), supporting that they may multimerize.

293 Based on the conservation of Ubi proteins, it is tempting to suggest that the UQ biosynthetic  
294 pathway of many proteobacteria is organized around a soluble stable metabolon, like in *E. coli*.  
295 Nonetheless, we expect different versions of the Ubi complex across proteobacteria because of  
296 variations in the number of UQ hydroxylases (Pelosi et al., 2016) and of other components of the  
297 pathway (Esposti, 2017). In eukaryotes, it is unclear whether a UQ biosynthesis metabolon exists since  
298 enzymes are still missing for two chemical reactions of the pathway (Awad et al., 2018). BN-PAGE  
299 experiments showed that several UQ biosynthetic proteins form a complex associated with the  
300 mitochondrial inner membrane in the yeast *S. cerevisiae* (He et al., 2014; Marbois et al., 2009), and  
301 such supramolecular organization may also exist in mammalian cells (Floyd et al., 2016; Stefely et al.,  
302 2016). However, the composition of the complex is uncertain since multiple proteins not related to UQ  
303 biosynthesis co-purified with yeast UQ biosynthetic proteins (Allan et al., 2015). Interestingly, two UQ  
304 biosynthesis proteins of unknown function and restricted to eukaryotes, Coq4 and Coq9, are able to

305 bind lipids (Lohman et al., 2014; Rea et al., 2010) and exhibit genetic interactions with other Coq  
306 proteins (Hsieh et al., 2007; Marbois et al., 2009; Ozeir et al., 2015). We suspect that Coq4 and Coq9  
307 may chaperone UQ intermediates, similar to bacterial UbiJ.

308 In conclusion, we discovered a stable metabolon - the Ubi complex - that catalyzes UQ  
309 biosynthesis, thanks to the association of five enzymes and two accessory factors. Surprisingly this  
310 metabolon localizes in the cytosolic fraction of *E. coli*, despite the extreme hydrophobicity of UQ  
311 biosynthetic intermediates. Our work shows that the SCP2 protein UbiJ binds these compounds and  
312 opens new questions regarding the trafficking of polyprenyl lipids in and out of membranes. The Ubi  
313 complex microreactor represents also a fascinating model to study how substrates are sequentially  
314 processed by multiple active sites located in a single structural entity.

315

## 316 SIGNIFICANCE

317 Ubiquinone is a conserved isoprenoid lipid of crucial physiological importance. Isoprenoid  
318 intermediates in ubiquinone biosynthesis are extremely hydrophobic; thus the pathway is proposed  
319 to be membrane-associated. Unexpectedly, we demonstrate that most steps in bacterial UQ  
320 biosynthesis are independent of the membrane and occur inside a soluble multiprotein complex that  
321 forms a stable metabolon. We elucidate a key role for the SCP2 domain of the UbiJ protein in binding  
322 the isoprenoid intermediates inside the multiprotein complex, documenting ~~that~~ the first example of  
323 the widespread SCP2 domain also functions in assisting chemical modifications of lipids. Our work  
324 highlights a remarkable bacterial metabolon synthesizing the hydrophobic ubiquinone molecule in the  
325 cytosol, and raises questions regarding the trafficking of isoprenoid lipids in and out of the membrane.

326

## 327 ACKNOWLEDGEMENTS

328 This work was supported by the Agence Nationale de la Recherche (ANR), ANR Blanc (An)aeroUbi ANR-  
329 15-CE11-0001-02. We thank Amélie Amblard and Mikael Martin for technical assistance and the GEM  
330 team at TIMC for discussions and suggestions. SB and YC thank the support of the discovery platform  
331 and data science group at EDyP. Proteomic experiments were partly supported by the Proteomics  
332 French Infrastructure (ANR-10-INBS-08-01 grant) and the Labex GRAL (ANR-10-LABX-49-01).  
333 Diffraction data were collected at the synchrotron SOLEIL, beamlines Proxima 1 and Proxima 2 (Saint-  
334 Aubin, France). We are most grateful to the beamline scientists, in particular Pierre Legrand and Bill  
335 Sheperd. CF, CDTV, ML and MF acknowledge support from the French National Research Agency (Labex  
336 program DYNAMO, ANR-11-LABX-0011) and from Fondation de l'Orangerie for Individual Philanthropy.

337

#### 338 **AUTHOR CONTRIBUTIONS**

339 FP, LP, MF, FB, ML, MHC designed research and FP coordinated the project. All authors performed  
340 experiments and/or data analysis. FP, LP, MF, FB, ML, and MHC wrote the paper and the manuscript  
341 was reviewed by all authors.

342

#### 343 **DECLARATION OF INTERESTS**

344 The authors declare no competing interests.

345

#### 346 **REFERENCES**

347 Alberge, F., Espinosa, L., Seduk, F., Sylvi, L., Toci, R., Walburger, A., and Magalon, A. (2015). Dynamic  
348 subcellular localization of a respiratory complex controls bacterial respiration. *Elife* 4.  
349 Alexander, K., and Young, I.G. (1978). Three hydroxylations incorporating molecular oxygen in the  
350 aerobic biosynthesis of ubiquinone in *Escherichia coli*. *Biochemistry* 17, 4745-4750.  
351 Allan, C.M., Awad, A.M., Johnson, J.S., Shirasaki, D.I., Wang, C., Blaby-Haas, C.E., Merchant, S.S., Loo,  
352 J.A., and Clarke, C.F. (2015). Identification of Coq11, a new coenzyme Q biosynthetic protein  
353 in the CoQ-synthome in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 290, 7517-7534.  
354 Aussel, L., Loiseau, L., Hajj Chehade, M., Pocachard, B., Fontecave, M., Pierrel, F., and Barras, F.  
355 (2014a). *ubij*, a New Gene Required for Aerobic Growth and Proliferation in Macrophage, *Is*



356 Involved in Coenzyme Q Biosynthesis in *Escherichia coli* and *Salmonella enterica* Serovar  
357 Typhimurium. *J. Bacteriol.* 196, 70-79.

358 Aussel, L., Pierrel, F., Loiseau, L., Lombard, M., Fontecave, M., and Barras, F. (2014b). Biosynthesis  
359 and physiology of coenzyme Q in bacteria. *Biochim. Biophys. Acta* 1837, 1004-1011.

360 Awad, A.M., Bradley, M.C., Fernandez-Del-Rio, L., Nag, A., Tsui, H.S., and Clarke, C.F. (2018).  
361 Coenzyme Q10 deficiencies: pathways in yeast and humans. *Essays Biochem.*

362 Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner,  
363 B.L., and Mori, H. (2006). Construction of *Escherichia coli* K-12 in-frame, single-gene knockout  
364 mutants: the Keio collection. *Mol. Syst. Biol.* 2, 2006 0008.

365 Babu, M., Butland, G., Pogoutse, O., Li, J., Greenblatt, J.F., and Emili, A. (2009). Sequential peptide  
366 affinity purification system for the systematic isolation and identification of protein  
367 complexes from *Escherichia coli*. *Methods Mol Biol* 564, 373-400.

368 Bader, M.W., Xie, T., Yu, C.A., and Bardwell, J.C. (2000). Disulfide bonds are generated by quinone  
369 reduction. *J. Biol. Chem.* 275, 26082-26088.

370 Bankaitis, V.A., Mousley, C.J., and Schaaf, G. (2010). The Sec14 superfamily and mechanisms for  
371 crosstalk between lipid metabolism and lipid signaling. *Trends Biochem. Sci.* 35, 150-160.

372 Barral, S., Morozumi, Y., Tanaka, H., Montellier, E., Govin, J., de Dieuleveult, M., Charbonnier, G.,  
373 Coute, Y., Puthier, D., Buchou, T., *et al.* (2017). Histone Variant H2A.L.2 Guides Transition  
374 Protein-Dependent Protamine Assembly in Male Germ Cells. *Mol. Cell* 66, 89-101 e108.

375 Bayly, C.L., and Yadav, V.G. (2017). Towards Precision Engineering of Canonical Polyketide Synthase  
376 Domains: Recent Advances and Future Prospects. *Molecules* 22, 18.

377 Bentinger, M., Tekle, M., and Dallner, G. (2010). Coenzyme Q-biosynthesis and functions. *Biochem.*  
378 *Biophys. Res. Commun.* 396, 74-79.

379 Bricogne, G., Blanc, E., Brandl, M., Flensburg, C., Keller, P., Paciorek, W., Roversi, P., Sharff, A., Smart,  
380 O.S., Vonrhein, C., *et al.* (2017). BUSTER version 2.10.2. Cambridge, United Kingdom: Global  
381 Phasing Ltd.

382 Burgardt, N.I., Gianotti, A.R., Ferreyra, R.G., and Ermacora, M.R. (2017). A structural appraisal of  
383 sterol carrier protein 2. *Biochimica Et Biophysica Acta-Proteins and Proteomics* 1865, 565-  
384 577.

385 Campanacci, V., Bishop, R.E., Blangy, S., Tegoni, M., and Cambillau, C. (2006). The membrane bound  
386 bacterial lipocalin Blc is a functional dimer with binding preference for lysophospholipids.  
387 *FEBS Lett.* 580, 4877-4883.

388 Carrica, M.C., Craig, P.O., Garcia-Angulo, V.A., Aguirre, A., Garcia-Vescovi, E., Goldbaum, F.A., and  
389 Cravero, S.L. (2011). YqjC of *Salmonella enterica* serovar Typhimurium is a membrane  
390 fusogenic protein required for mice colonization. *BMC Microbiol* 11, 95.

391 Cheng, W., and Li, W. (2014). Structural insights into ubiquinone biosynthesis in membranes. *Science*  
392 343, 878-881.

393 Chovancova, E., Pavelka, A., Benes, P., Strnad, O., Brezovsky, J., Kozlikova, B., Gora, A., Sustr, V.,  
394 Klvana, M., Medek, P., *et al.* (2012). CAVER 3.0: a tool for the analysis of transport pathways  
395 in dynamic protein structures. *PLoS Comput. Biol.* 8, e1002708.

396 Cowtan, K. (2006). The Buccaneer software for automated model building. 1. Tracing protein chains.  
397 *Acta Crystallogr D Biol Crystallogr* 62, 1002-1011.

398 De Berti, F.P., Capaldi, S., Ferreyra, R., Burgardt, N., Acierno, J.P., Klinke, S., Monaco, H.L., and  
399 Ermacora, M.R. (2013). The crystal structure of sterol carrier protein 2 from *Yarrowia*  
400 *lipolytica* and the evolutionary conservation of a large, non-specific lipid-binding cavity. *J.*  
401 *Struct. Funct. Genomics* 14, 145-153.

402 Dyer, D.H., Wessely, V., Forest, K.T., and Lan, Q. (2008). Three-dimensional structure/function  
403 analysis of SCP-2-like2 reveals differences among SCP-2 family members. *J. Lipid Res.* 49, 644-  
404 653.

405 Emsley, P., Lohkamp, B., Scott, W.G., and Cowtan, K. (2010). Features and development of Coot. *Acta*  
406 *Crystallogr D Biol Crystallogr* 66, 486-501.

407 Esposti, M.D. (2017). A journey across genomes uncovers the origin of ubiquinone in cyanobacteria.  
408 Genome Biol Evol.

409 Floyd, B.J., Wilkerson, E.M., Veling, M.T., Minogue, C.E., Xia, C., Beebe, E.T., Wrobel, R.L., Cho, H.,  
410 Kremer, L.S., Alston, C.L., *et al.* (2016). Mitochondrial Protein Interaction Mapping Identifies  
411 Regulators of Respiratory Chain Function. *Mol. Cell* 63, 621-632.

412 Galassi, V.V., and Arantes, G.M. (2015). Partition, orientation and mobility of ubiquinones in a lipid  
413 bilayer. *Biochimica Et Biophysica Acta-Bioenergetics* 1847, 1560-1573.

414 Gangar, A., Karande, A.A., and Rajasekharan, R. (2001). Isolation and localization of a cytosolic 10 S  
415 triacylglycerol biosynthetic multienzyme complex from oleaginous yeast. *J. Biol. Chem.* 276,  
416 10290-10298.

417 Haapalainen, A.M., van Aalten, D.M., Merilainen, G., Jalonen, J.E., Pirila, P., Wierenga, R.K., Hiltunen,  
418 J.K., and Glumoff, T. (2001). Crystal structure of the liganded SCP-2-like domain of human  
419 peroxisomal multifunctional enzyme type 2 at 1.75 Å resolution. *J. Mol. Biol.* 313, 1127-1138.

420 Hajj Chehade, M., Loiseau, L., Lombard, M., Pecqueur, L., Ismail, A., Smadja, M., Golinelli-Pimpaneau,  
421 B., Mellot-Draznieks, C., Hamelin, O., Aussel, L., *et al.* (2013). *ubil*, a New Gene in *Escherichia*  
422 *coli* Coenzyme Q Biosynthesis, Is Involved in Aerobic C5-hydroxylation. *J. Biol. Chem.* 288,  
423 20085-20092.

424 He, C.H., Xie, L.X., Allan, C.M., Tran, U.C., and Clarke, C.F. (2014). Coenzyme Q supplementation or  
425 over-expression of the yeast Coq8 putative kinase stabilizes multi-subunit Coq polypeptide  
426 complexes in yeast coq null mutants. *Biochim. Biophys. Acta* 1841, 630-644.

427 Hoyo, J., Gaus, E., and Torrent-Burgues, J. (2017). Tuning ubiquinone position in biomimetic  
428 monolayer membranes. *European Physical Journal E* 40.

429 Hsieh, E.J., Gin, P., Gulmezian, M., Tran, U.C., Saiki, R., Marbois, B.N., and Clarke, C.F. (2007).  
430 *Saccharomyces cerevisiae* Coq9 polypeptide is a subunit of the mitochondrial coenzyme Q  
431 biosynthetic complex. *Arch. Biochem. Biophys.* 463, 19-26.

432 Hu, P.Z., Janga, S.C., Babu, M., Diaz-Mejia, J.J., Butland, G., Yang, W.H., Pogoutse, O., Guo, X.H.,  
433 Phanse, S., Wong, P., *et al.* (2009). Global Functional Atlas of *Escherichia coli* Encompassing  
434 Previously Uncharacterized Proteins. *PLoS Biol.* 7, 929-947.

435 Huang, H., Levin, E.J., Liu, S., Bai, Y., Lockless, S.W., and Zhou, M. (2014). Structure of a Membrane-  
436 Embedded Prenyltransferase Homologous to UBIAD1. *PLoS Biol.* 12, e1001911.

437 Kabsch, W. (2010). Xds. *Acta Crystallogr D Biol Crystallogr* 66, 125-132.

438 Karimova, G., Pidoux, J., Ullmann, A., and Ladant, D. (1998). A bacterial two-hybrid system based on a  
439 reconstituted signal transduction pathway. *Proc. Natl. Acad. Sci. U. S. A.* 95, 5752-5756.

440 Kawamukai, M. (2018). Biosynthesis and applications of prenylquinones. *Biosci. Biotechnol. Biochem.*  
441 82, 963-977.

442 Knoell, H.E. (1979a). Isolation of a soluble enzyme complex comprising the ubiquinone-8 synthesis  
443 apparatus from the cytoplasmic membrane of *Escherichia coli*. *Biochem. Biophys. Res.*  
444 *Commun.* 91, 919-925.

445 Knoell, H.E. (1979b). Ubiquinone synthesis in vitro starting from 2-octaprenyl phenol. *FEBS Lett.* 97,  
446 155-158.

447 Laursen, T., Borch, J., Knudsen, C., Bavishi, K., Torta, F., Martens, H.J., Silvestro, D., Hatzakis, N.S.,  
448 Wenk, M.R., Dafforn, T.R., *et al.* (2016). Characterization of a dynamic metabolon producing  
449 the defense compound dhurrin in sorghum. *Science* 354, 890-893.

450 Li, G.W., Burkhardt, D., Gross, C., and Weissman, J.S. (2014). Quantifying absolute protein synthesis  
451 rates reveals principles underlying allocation of cellular resources. *Cell* 157, 624-635.

452 Lohman, D.C., Forouhar, F., Beebe, E.T., Stefely, M.S., Minogue, C.E., Ulbrich, A., Stefely, J.A.,  
453 Sukumar, S., Luna-Sanchez, M., Jochem, A., *et al.* (2014). Mitochondrial COQ9 is a lipid-  
454 binding protein that associates with COQ7 to enable coenzyme Q biosynthesis. *Proc. Natl.*  
455 *Acad. Sci. U. S. A.* 111, E4697-4705.

456 Loiseau, L., Fyfe, C., Aussel, L., Hajj Chehade, M., Hernandez, S.B., Faivre, B., Hamdane, D., Mellot-  
457 Draznieks, C., Rascalou, B., Pelosi, L., *et al.* (2017). The UbiK protein is an accessory factor

458 necessary for bacterial ubiquinone (UQ) biosynthesis and forms a complex with the UQ  
 459 biogenesis factor UbiI. *J. Biol. Chem.* *292*, 11937-11950.  
 460 Lopez-Lara, I.M., and Geiger, O. (2017). Bacterial lipid diversity. *Biochimica Et Biophysica Acta-*  
 461 *Molecular and Cell Biology of Lipids* *1862*, 1287-1299.  
 462 Marbois, B., Gin, P., Gulmezian, M., and Clarke, C.F. (2009). The yeast Coq4 polypeptide organizes a  
 463 mitochondrial protein complex essential for coenzyme Q biosynthesis. *Biochim. Biophys.*  
 464 *Acta* *1791*, 69-75.  
 465 Nguyen, C., Haushalter, R.W., Lee, D.J., Markwick, P.R.L., Bruegger, J., Caldara-Festin, G., Finzel, K.,  
 466 Jackson, D.R., Ishikawa, F., O'Dowd, B., *et al.* (2014). Trapping the dynamic acyl carrier  
 467 protein in fatty acid biosynthesis. *Nature* *505*, 427-431.  
 468 Nowicka, B., and Kruk, J. (2010). Occurrence, biosynthesis and function of isoprenoid quinones.  
 469 *Biochim. Biophys. Acta* *1797*, 1587-1605.  
 470 Ozeir, M., Pelosi, L., Ismail, A., Mellot-Draznieks, C., Fontecave, M., and Pierrel, F. (2015). Coq6 Is  
 471 Responsible for the C4-deamination Reaction in Coenzyme Q Biosynthesis in *Saccharomyces*  
 472 *cerevisiae*. *J. Biol. Chem.* *290*, 24140-24151.  
 473 Pavelka, A., Sebestova, E., Kozlikova, B., Brezovsky, J., Sochor, J., and Damborsky, J. (2016). CAVER:  
 474 Algorithms for Analyzing Dynamics of Tunnels in Macromolecules. *IEEE/ACM Trans Comput*  
 475 *Biol Bioinform* *13*, 505-517.  
 476 Pelosi, L., Ducluzeau, A.L., Loiseau, L., Barras, F., Schneider, D., Junier, I., and Pierrel, F. (2016).  
 477 Evolution of Ubiquinone Biosynthesis: Multiple Proteobacterial Enzymes with Various  
 478 Regioselectivities To Catalyze Three Contiguous Aromatic Hydroxylation Reactions. *mSystems*  
 479 *1*, e00091-00016.  
 480 Poon, W.W., Davis, D.E., Ha, H.T., Jonassen, T., Rather, P.N., and Clarke, C.F. (2000). Identification of  
 481 *Escherichia coli* ubiB, a gene required for the first monooxygenase step in ubiquinone  
 482 biosynthesis. *J. Bacteriol.* *182*, 5139-5146.  
 483 Rea, S.L., Graham, B.H., Nakamaru-Ogiso, E., Kar, A., and Falk, M.J. (2010). Bacteria, yeast, worms,  
 484 and flies: exploiting simple model organisms to investigate human mitochondrial diseases.  
 485 *Dev. Disabil. Res. Rev.* *16*, 200-218.  
 486 Reidenbach, A.G., Kemmerer, Z.A., Aydin, D., Jochem, A., McDevitt, M.T., Hutchins, P.D., Stark, J.L.,  
 487 Stefely, J.A., Reddy, T., Hebert, A.S., *et al.* (2017). Conserved Lipid and Small-Molecule  
 488 Modulation of COQ8 Reveals Regulation of the Ancient Kinase-like UbiB Family. *Cell Chem*  
 489 *Biol.*  
 490 Schoepp-Cothenet, B., van Lis, R., Atteia, A., Baymann, F., Capowiez, L., Ducluzeau, A.L., Duval, S., ten  
 491 Brink, F., Russell, M.J., and Nitschke, W. (2013). On the universal core of bioenergetics.  
 492 *Biochim. Biophys. Acta* *1827*, 79-93.  
 493 Sheldrick, G.M. (2015). Crystal structure refinement with SHELXL. *Acta Crystallogr C Struct Chem* *71*,  
 494 3-8.  
 495 Shestopalov, A.I., Bogachev, A.V., Murtazina, R.A., Viryasov, M.B., and Skulachev, V.P. (1997).  
 496 Aeration-dependent changes in composition of the quinone pool in *Escherichia coli* -  
 497 Evidence of post-transcriptional regulation of the quinone biosynthesis. *FEBS Lett.* *404*, 272-  
 498 274.  
 499 Srere, P.A. (1985). THE METABOLON. *Trends Biochem. Sci.* *10*, 109-110.  
 500 Stefely, J.A., Licitra, F., Laredj, L., Reidenbach, A.G., Kemmerer, Z.A., Grangeray, A., Jaeg-Ehret, T.,  
 501 Minogue, C.E., Ulbrich, A., Hutchins, P.D., *et al.* (2016). Cerebellar Ataxia and Coenzyme Q  
 502 Deficiency through Loss of Unorthodox Kinase Activity. *Mol. Cell* *63*, 608-620.  
 503 Stefely, J.A., and Pagliarini, D.J. (2017). Biochemistry of Mitochondrial Coenzyme Q Biosynthesis.  
 504 *Trends Biochem. Sci.* *42*, 824-843.  
 505 Su, G., Morris, J.H., Demchak, B., and Bader, G.D. (2014). Biological network exploration with  
 506 Cytoscape 3. *Curr Protoc Bioinformatics* *47*, 8 13 11-24.  
 507 Winn, M.D., Ballard, C.C., Cowtan, K.D., Dodson, E.J., Emsley, P., Evans, P.R., Keegan, R.M., Krissinel,  
 508 E.B., Leslie, A.G., McCoy, A., *et al.* (2011). Overview of the CCP4 suite and current  
 509 developments. *Acta Crystallogr D Biol Crystallogr* *67*, 235-242.

510 Wittig, I., Braun, H.P., and Schagger, H. (2006). Blue native PAGE. *Nat. Protoc.* *1*, 418-428.  
511 Wollstein, C., Winterhalter, M., and Funari, S.S. (2015). The location of coenzyme Q10 in  
512 phospholipid membranes made of POPE: a small-angle synchrotron X-ray diffraction study.  
513 *European Biophysics Journal with Biophysics Letters* *44*, 373-381.  
514 Wu, F., and Minter, S. (2015). Krebs Cycle Metabolon: Structural Evidence of Substrate Channeling  
515 Revealed by Cross-Linking and Mass Spectrometry. *Angewandte Chemie-International*  
516 *Edition* *54*, 1851-1854.  
517 Xia, H., Yang, X., Tang, Q., Ye, J., Wu, H., and Zhang, H. (2017). EsrE-A yigP Locus-Encoded Transcript-  
518 Is a 3' UTR sRNA Involved in the Respiratory Chain of *E. coli*. *Front Microbiol* *8*, 1658.  
519 Yu, L.J., Suga, M., Wang-Otomo, Z.Y., and Shen, J.R. (2018). Structure of photosynthetic LH1-RC  
520 supercomplex at 1.9 Å resolution. *Nature* *556*, 209-213.  
521 Zhou, Z.H., McCarthy, D.B., O'Connor, C.M., Reed, L.J., and Stoops, J.K. (2001). The remarkable  
522 structural and functional organization of the eukaryotic pyruvate dehydrogenase complexes.  
523 *Proc. Natl. Acad. Sci. U. S. A.* *98*, 14802-14807.

524  
525

**Commented [FP1]:** Insertion of one extra reference : Baba et al 2006 because the reference is cited in Table S5

526 **Figure legends:**

527 **Figure 1: UQ biosynthesis activity and Ubi proteins localize in the soluble fraction of *E. coli* extracts.**

528 A) UQ biosynthesis pathway in *E. coli*. The octaprenyl tail is represented by R on the biosynthetic  
529 intermediates. Abbreviations used are DMAPP for dimethylallyl pyrophosphate, IPP for isopentenyl  
530 pyrophosphate, 4-HB for 4-hydroxybenzoic acid, DDMQ<sub>8</sub> for C2-demethyl-C6-demethoxy-ubiquinone  
531 8, DMQ<sub>8</sub> for C6-demethoxy-ubiquinone 8 and UQ<sub>8</sub> for ubiquinone 8. B) Synthesis of <sup>13</sup>C<sub>6</sub>-UQ<sub>8</sub> from <sup>13</sup>C<sub>6</sub>-  
532 OPP accumulated in  $\Delta ubiC$  cells. Cell free extracts, membrane and soluble fractions (resulting from  
533 150.000g ultracentrifugation) were supplemented with NADH and SAM and incubated at 30°C in  
534 aerobic conditions for the indicated time. The area of the <sup>13</sup>C-UQ<sub>8</sub> peak (m/z 764.5 at a retention time  
535 of 8 min., see Figure S1E) was normalized for the protein content of the fractions assayed. Results  
536 representative of three independent experiments. C) Immunodetection of Ubi-SPA proteins ( $\alpha$ -FLAG  
537 antibody) in soluble (s) and membrane (m) fractions of Ubi-SPA tagged strains (30  $\mu$ g of total proteins)  
538 separated by SDS-PAGE. LamB and GAPDH are detected as controls. The ratio of the total content of  
539 each Ubi protein in entire soluble and membrane fractions is shown at the bottom (see Method  
540 Details). See also Figure S1.

541

542 **Figure 2: Seven Ubi proteins form a soluble Ubi complex.**

543 A) BN-PAGE electrophoresis and immunodetection of soluble fractions from the indicated Ubi-SPA strains (50  $\mu$ g of total proteins). The  
544 membrane was first probed with  $\alpha$ -FLAG antibody, stripped and probed with  $\alpha$ -GAPDH. B)  
545 Immunodetection with  $\alpha$ -FLAG antibody of soluble fractions from the indicated Ubi-SPA strains after  
546 1D BN-PAGE and 2D SDS-PAGE (50  $\mu$ g of total proteins). C) SDS-PAGE and western-blot analysis ( $\alpha$ -  
547 FLAG antibody) of fractions recovered from glycerol gradients onto which the cytoplasm of the  
548 indicated Ubi-SPA cells were deposited. A typical distribution of GAPDH is also shown. Fractions 1 and  
549 22 correspond to the top and the bottom of the gradient, respectively (densities 1.02 and 1.11). Silver  
550 staining of gradient fractions analyzed by SDS-PAGE shows the distribution of total proteins (bottom).  
551 D) Separation by 1D BN-PAGE and 2D SDS-PAGE of glycerol gradient fractions 13-15 containing UbiG-  
552 SPA and immunodetection with  $\alpha$ -FLAG antibody. The distribution of UbiG in all fractions of the  
553 gradient is shown (top). E) Interaction network between Ubi proteins constructed with Cytoscape 3  
554 from the results of the bacterial two hybrid experiments (Table S1). Line width is proportional to the  
555 value of the interaction and cut off value was three times background. Interaction of proteins with  
556 themselves (for example UbiA-UbiA) are not represented. Results representative of two independent  
557 experiments (A-C). See also Figure S2.

558

559 **Figure 3: Purification of the Ubi complex.**

560 A) Immunodetection with  $\alpha$ -CBP antibody of the purified  
561 Ubi complex after 1D BN-PAGE and 2D SDS-PAGE (E2 fraction of UbiE-SPA, see Figure S2F). B) Silver  
562 staining of SDS-PAGE with purified E2 fractions from indicated -SPA samples and WT controls. Asterisks  
563 mark bands common to -SPA samples and absent from WT. C) Venn diagram integrating the different  
564 proteins found to be specifically enriched with each bait as compared with the related negative  
565 controls using MS-based proteomics (Table S2). The names of the eight proteins enriched with all three  
tagged Ubi proteins are indicated. See also Figure S2.

566

567 **Figure 4: The SCP2 domain of UbiJ binds UQ<sub>8</sub> and its biosynthetic intermediates in the Ubi complex.**  
568 A) Ratio of the abundance of octaprenyl lipids in the soluble fraction (SF, 150.000g supernatant) and  
569 the membrane fraction (MF) of WT cells grown in LB medium, mean  $\pm$ SD (n=6). B) Glycerol gradient  
570 analysis of soluble extracts from UbiH-SPA cells and quantification of isoprenoid quinones in each  
571 fraction by HPLC-ECD-MS. Recovery calculated from the total content of all fractions compared to  
572 content of the UbiH-SPA soluble fraction deposited on top of the gradient. The western-blot showing  
573 the position of UbiH in the gradient is the same than in Figure 2C. C) Levels of OPP, DMQ<sub>8</sub> and UQ<sub>8</sub> in  
574 cells overexpressing or not UbiJ-K (n=4), see Figure S3C, \*\*: p< 0.01, \*\*\*: p< 0.001, unpaired Student's  
575 t test. D) Abundance of isoprenoid quinones in the indicated purified proteins (see Figure S3E), mean  
576  $\pm$ SEM (n=2). See also Figure S3.

577

578 **Figure 5. Structure of UbiJ-SCP2 at 1.7 Å resolution.** A) UbiJ-SCP2 appearing as a dimer within the  
579 asymmetric unit, monomers contain a beta sheet consisting of 5 strands and 5 alpha helices similar to  
580 SCP2 family proteins (chain A in orange, chain B in teal). B) Yellow spheres represent the cavity found  
581 in UbiJ-SCP2 (cavity modelled using CAVER3 plugin for Pymol). C) Model of the cavity area of UbiJ-SCP2  
582 with the hydrophobic residues lining the cavity highlighted in yellow and showing unmodelled electron  
583 density (maps for 2FoFc are set at 1  $\sigma$  [blue mesh] and for FoFc are set at 3  $\sigma$  [green mesh]). D) Sequence  
584 for UbiJ-SCP2 with hydrophobic residues interfacing with the cavity pocket shown in yellow (cylinders  
585 represent  $\alpha$ -helices, arrows  $\beta$ -strands). E) Hydrophobic cavities in UbiJ-SCP2 chain A (top) and chain B  
586 (middle), and comparison with ubiquinone 8 (bottom, taken from PDB: 5Y5S). The respective lengths  
587 are indicated. See also Figure S4 and S5.

588

589 **Figure 6: UbiJ is essential to the stability of the Ubi complex.** A) Glycerol gradient analysis of soluble  
590 extracts from UbiG-SPA  $\Delta$ *ubiJ* cells and quantification of UQ<sub>8</sub> and OPP in each fraction. Western-blot  
591 show the distribution of UbiG-SPA and the control GAPDH protein (see also Figure S6C-D, recovery  
592 calculated as in Figure 4B). B-C) Immunodetection with  $\alpha$ -FLAG antibody of soluble fractions from the  
593 UbiH-SPA strain carrying deletions in the indicated *ubi* genes after BN-PAGE analysis (B) or 1D BN-PAGE  
594 and 2D SDS-PAGE (C). D-E) Immunodetection with  $\alpha$ -FLAG antibody of soluble fractions from the  
595 indicated Ubi-SPA strain carrying deletions for  $\Delta$ *ubiJ* (D) or  $\Delta$ *ubiK* (E) after 1D BN-PAGE and 2D SDS-  
596 PAGE. A representative result from 2 independent experiments is shown (B-E). F) UQ<sub>8</sub> biosynthetic  
597 pathway (top) and proposed model of the physical organization of Ubi proteins and prenylated UQ<sub>8</sub>  
598 intermediates with respect to the inner membrane. The same representation is used for all  
599 polyprenylated compounds, irrespective of the substituents of the benzoquinone head group.  
600 Membrane-bound UbiA prenylates 4HB to yield OHB that localizes in the lipid bilayer. UbiB assists the  
601 extraction of OHB from the membrane and OHB is subsequently decarboxylated to OPP by UbiD-X.  
602 OPP is bound by UbiJ in the Ubi complex and the metabolon formed by the seven Ubi proteins circled  
603 in red synthesizes UQ<sub>8</sub>, which is ultimately delivered to the membrane, where it fulfills its physiological  
604 functions. See also Figure S6.

605

606 **STAR Methods**

607 **CONTACT FOR REAGENT AND RESOURCE SHARING**

608 Further information and requests for resources and reagents should be directed to and will be  
609 fulfilled by the Lead Contact, Fabien Pierrel ([fabien.pierrel@univ-grenoble-alpes.fr](mailto:fabien.pierrel@univ-grenoble-alpes.fr))

610 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

611 *E. coli* strains were stored frozen at -80°C in solutions containing 20% glycerol and revived by streaking  
612 on LB plates containing 1.6% bacto-agar (w/v) and incubating overnight at 37°C. Plates were kept at  
613 4°C for up to three weeks and colonies from the plates were used to inoculate overnight precultures  
614 in liquid medium. Large cultures were performed by inoculating fresh growth medium at O<sub>D600</sub> 0.05  
615 with the precultures. *E. coli* strains were grown in lysogeny broth (LB) medium at 37 °C in Erlenmeyer  
616 flasks filled to 1/10 of the maximal volume and shaken at 180 rpm, unless stated otherwise. For  
617 experiments using labelling with <sup>13</sup>C<sub>7</sub>-4-HB, cells were grown in MSG medium, a minimal medium  
618 supplemented with 0.4% glycerol (w/v) (Alberge et al., 2015). All growth media were sterilized by  
619 autoclave and then ampicillin (50 mg/L), kanamycin (25 mg/L), and chloramphenicol (25 mg/L) were  
620 added from stocks (1000X solution sterilized through 0.22 µm filters and stored at -20°C), when  
621 needed. MSG medium was supplemented with 0.5% casaminoacids (w/v) and with 1/100 volume of a  
622 filter-sterile solution of 1mM CaCl<sub>2</sub>, 200 mM MgCl<sub>2</sub>, 1% thiamine (w/v).

623 **METHOD DETAILS**

624 ***Strain construction:***

625 All strains used in this study are listed in Table S5.

626 Construction of SPA strains:

627 The DY330 *ubiC-SPA* strain encompassing the SPA tag DNA sequence and the kanamycin antibiotic  
628 resistance marker cassette (Kan<sup>R</sup>) was used as a template in polymerase chain reaction (PCR)  
629 amplification. A 5-*ubiD*, *E*, *I*, *J*, *K* -SPA gene-specific forward primer (Table S6), located immediately

630 upstream of the *ubi* stop codon, and a 3-*ubiD*, *E*, *I*, *J*, *K* -SPA gene-specific reverse primer, located  
631 immediately downstream of the target gene stop codon, were used to amplify the SPA tag and Kan<sup>R</sup>  
632 cassette. The purified PCR products were subsequently integrated at the 3' end of *ubi* genes or *atpB*  
633 gene in the BW25113 strain in which the  $\lambda$ -Red recombination machinery is expressed. Finally, the *ubi*-  
634 SPA-Kan cassettes were transduced through P1 phage from BW25113 to MG1655. When DY330 SPA-  
635 tagged strains were available (Hu et al., 2009), the *ubi*-SPA-Kan cassettes were transduced to MG1655  
636 through P1 phage.

637 Construction of knock-out strains:

638 The *ubiC*::Kan, *ubiE*::Kan, *ubiF*::Kan, *ubiG*::Kan mutations (from the BW25113 strains, Keio  
639 library) and *ubiJ*::Kan, *ubiK*::Kan mutations were transduced into the desired MG1655 strain by P1  
640 transduction.

641 **Plasmid construction:**

642 The two-hybrid plasmids were constructed by inserting the *ubi* ORF sequences in pT18 and pT25  
643 plasmids, using the oligonucleotides indicated in Table S6. Briefly, the *ubi* gene fragments were  
644 obtained by PCR amplification using the genomic DNA of MG1655 as template and the oligonucleotides  
645 5-*XbaI* *ubi* and 3-*XbaI* *ubi*. PCR products were *XbaI*-digested and inserted into *XbaI*-digested pT18 or  
646 pT25 plasmids, yielding the pT18-*ubi* and pT25-*ubi* plasmids, respectively. All plasmids were verified  
647 using DNA sequencing.

648 **Bacterial two-hybrid reporter system:**

649 The bacterial two-hybrid reporter system is based on functional complementation of the two domains  
650 of *Bordetella pertussis* adenylate cyclase, T18 and T25, expressed separately from two compatible  
651 plasmids (Karimova et al., 1998). Adenylate cyclase activity is restored only when proteins fused to T18  
652 and T25 interact. Fusions were constructed with T18 and T25 present at the N-terminal of Ubi proteins.  
653 Functional reconstitution of *B. pertussis* adenylate cyclase in an *E. coli* *\_cya lac\_* strain was monitored  
654 by assaying the activity of a cAMP-CRP-dependent lac promoter of a chromosomally encoded lac



655 operon. In addition to testing the pair of proteins of interest, positive controls were performed with  
656 T25-zip and T18-zip plasmids. Negative controls were performed with the two empty T18 and T25  
657 plasmids and also with each protein of interest against the empty T18 or T25 plasmids.

658 The interaction network of Ubi proteins in Figure 1E was constructed with Cytoscape 3 (Su et al., 2014)  
659 from the results of the bacterial two hybrid experiments (Table S1), excluding values less than 3 times  
660 above background.

661 ***Preparation of soluble and membrane fractions:***

662 Ubi-SPA strains were cultured in 1 L of LB medium (2 L flasks) with shaking (200 rpm) at 37°C. When  
663 OD<sub>600</sub> reached 1.5-2, cells were cooled down on ice with occasional shaking and harvested by  
664 centrifugation at 5,000 g for 20 min at 4°C. The cell pellet was washed with 10 mL cold water, the same  
665 centrifugation was repeated and the pellet was frozen in liquid nitrogen, then stored at -80°C. The cell  
666 pellet was resuspended in 30 mL buffer A (50 mM Imidazole/HCl pH=7, 50 mM NaCl, 1 mM EDTA, 1  
667 mM PMSF, 250 mM sucrose; (adapted from (Wittig et al., 2006))) and the cells were ruptured by 3  
668 passages through French press (1,500 psi). Cell debris were eliminated by centrifugation at 3,200 g for  
669 10 min at 4°C, then 20 mL of the supernatant was subjected to ultracentrifugation at 120,000 g for 1h  
670 at 4°C. The supernatant after ultracentrifugation corresponds to the soluble fraction. The pellet was  
671 resuspended in 2 mL of buffer A to yield the membrane fraction. Aliquots of both fractions were frozen  
672 with liquid nitrogen and stored at -80°C.

673 ***Protein concentration and SDS-PAGE:***

674 Protein concentrations were determined by the bicinchoninic acid assay (Thermo). 30 µg of total  
675 protein were analyzed on 12% acrylamide SDS-PAGE gel and electrophoresis was performed at 20 mA  
676 for 45 min. Prestained Protein Ladder (Euromedex) was used as molecular weight marker.

677 ***Blue Native-PAGE:***

678 Native PAGE sample additive (Life Technologies) was added to the soluble fractions (50 µg of proteins)  
679 and BN-PAGE was performed using Native PAGE 3–12% Bis–Tris gels (1.0 mm × 10 wells; Life

680 Technologies). The BN-PAGE system consisted of two compartments: the internal one contained the  
681 cathode buffer (50 mM tricine, 7.5 mM imidazole, 0.02% coomassie blue G250, pH=7) whereas the  
682 external one contained the NativePAGE running buffer (50 mM Bis-Tris pH=7, 50 mM tricine, Life  
683 Technologies). Electrophoresis was performed at 4°C using 3 tensions: 100V for 20 min allowing  
684 proteins to enter the gel, 200V for 30 min then 300V until the migration front reached the lower limit  
685 of the gel (~1h). Proteins were transferred onto an immobilon PVDF membrane pre-activated for 2 min  
686 with methanol. Transfer was performed in buffer B (50 mM Tricine, 7.5 mM imidazole, pH=7) for 3h at  
687 4°C with 20 mV tension. At the end of the transfer, the PVDF membrane was discolored by rinsing  
688 several times with a mixture of 20% methanol and 10% acetic acid in order to visualize the molecular  
689 weight marker (NativeMark, Life Technologies). The position of marker bands on the membrane was  
690 marked with pencil and full discoloration of the membrane was obtained with 100% methanol. The  
691 membrane was further processed for western-blotting.

692 ***Two dimensional BN-PAGE/SDS-PAGE:***

693 After the BN-PAGE was completed, gel lanes were cut out of the gel, soaked in SDS-PAGE running  
694 buffer (0.375 M Tris-HCl, pH 8.8, glycine 0.192 M, SDS 10%) for 15 min and then loaded onto a 12.5%  
695 acrylamide SDS gel of 1.5 mm thickness. 5 µL of molecular weight marker (prestained protein ladder)  
696 was deposited on a Whatman paper, which was positioned next to the BN-PAGE fragment. BN gel and  
697 SDS gel were glued together using buffer C (0.5% agarose dissolved in SDS running buffer and few  
698 drops of 0.5% bromophenol blue). Migration was performed at 20 mA until the migration front reached  
699 the bottom of the gel. Transfer was performed using trans-blot turbo system (Bio-Rad) 17 V, 10 min.

700 ***Western-blot analyses:***

701 After transfer, membranes were saturated with 5% milk in PBST (Phosphate-buffered saline, 0.2%  
702 Tween-20) for 30 min, rinsed 3 times in PBST and incubated overnight with the primary antibody in  
703 PBST at 4°C on a rotary table. Primary antibodies were used at the following dilutions: α-FLAG antibody,  
704 1/5000; α-CBP, 1/3000; α-GAPDH, 1/5000; α-LamB, 1/3000. Immunodetection was performed using  
705 goat anti-rabbit HRP-conjugated or goat anti-mouse HRP-conjugated and the Clarity Western ECL

706 substrate (BioRad). Images were acquired on a ChemiDoc XRS system with the Image Lab software  
707 (BioRad). When detection with a second antibody was needed, the membrane was stripped by three  
708 successive incubations in stripping buffer (glycine-HCl 1.5% (m/V) pH 2.2, SDS 0.1% (m/V), Tween 1%  
709 (V/V)), then rinsed in PBST and saturated with 5% milk in PBST.

710 The s/m ratio in Figure 1C were calculated as follows. The intensity of each protein band (quantified  
711 from the western-blot with Image Lab software) was multiplied by the total protein content of the  
712 corresponding fraction (typically, 18 mL at ~5 mg protein/mL for FS and 2 mL at ~19 mg protein/mL  
713 for FM) to estimate the total content of Ubi-SPA proteins in s and m fractions. Then, we computed the  
714 quotient of the values obtained for s and m for each Ubi-SPA protein.

715

#### 716 ***Silver staining:***

717 The SDS-gel was placed in fixing solution (50% methanol, 5% acetic acid) and agitated gently on a rotary  
718 shaker for 20 min. The gel was then rinsed in 50% methanol for 10 min, in water for 10 min and was  
719 agitated gently in 100 mL of 0.02% sodium-thiosulfate for 1 min, followed by two washing steps with  
720 water for 1 min. The gel was incubated for 20 min in 200 mL of 0.2% silver nitrate solution  
721 supplemented with 0.08% formaldehyde. The gel was washed with distilled water for 20 sec to remove  
722 the excess silver nitrate and was then placed in 50 mL of developing solution (2% sodium carbonate  
723 and 0.04% formaldehyde). When the desired intensity was obtained, the gel was transferred to 5%  
724 acetic acid for 20 min to stop the reaction. After rinsing with distilled water for 10 min, the gel was  
725 stored in 5% glycerol.

#### 726 ***Overexpression and purification of proteins:***

727 The UbiJ(6his)-UbiK and UbiK proteins were overexpressed and purified as previously described  
728 (Loiseau et al., 2017). Overexpression of UbiJ-SCP2 was performed in BL21 (DE3) *E.coli* strain  
729 transformed with the previously reported pETDuet-ubiJ( $\Delta$ 121–201) (Loiseau et al., 2017). Cells were  
730 grown with shaking (200 rpm) at 37°C, in lysogeny broth (LB) medium containing ampicillin (100  
731  $\mu$ g/ml), until they reached  $OD_{600} = 0.4$ , at which point overexpression was induced by addition of

732 lactose (5.8 mM). After induction, cells were grown for 17 hours at 16°C, and then harvested by  
733 centrifugation at 5,000 g for 10 min. All subsequent operations were carried out at 4°C. Cells were  
734 resuspended in 5 volumes of buffer D (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM  
735 phenylmethylsulfonyl fluoride) and lysed by sonication (Branson Digital Sonifier, Amplitude 40% for 10  
736 min). Crude extracts were then submitted to ultracentrifugation at 180,000 g for 90 min (Optima XPN-  
737 80, rotor 50.2 Ti, Beckman Coulter). The resulting supernatant was loaded onto a Hitrap Chelating HP  
738 column (5 mL, GE Healthcare) equilibrated with buffer D. The Hitrap Chelating HP column was washed  
739 with buffer D supplemented with 25 mM imidazole and subsequently eluted with 300 mM imidazole  
740 in buffer D. The most pure Ubij-SCP2 fractions were pooled and loaded onto a HiLoad 26/600 Superdex  
741 75 pg equilibrated with 50 mM Tris-HCl pH7.5, 150 mM NaCl. The main peak fractions were pooled,  
742 frozen in liquid N2 and stored at - 80°C.

743 **Crystallization:**

744 Crystals were grown at 19 °C by the hanging drop vapor diffusion method. Initial reservoir  
745 crystallization hit consisted of 18% PEG 8000, 200 mM calcium acetate hydrate with 100 mM sodium  
746 cacodylate trihydrate pH 6.5. Crystal growth was observed in high protein concentration of no less  
747 than 15 mg/mL. The reservoir condition was optimized for best crystal growth to consist of 11% PEG  
748 8000, 225 mM calcium chloride with 100 mM MMT buffer pH 9 (MMT is DL-malic acid/MES/Tris base  
749 mixed at a ratio of 1/2/2 and pH-adjusted with sodium hydroxide). Rod-like crystals grew within the  
750 precipitate overnight. All crystals were cryoprotected in a solution similar to the reservoir solution  
751 containing 20 % PEG 400 prior to flash freezing. Best diffraction of these crystals led to the initial 2.5 Å  
752 dataset (PDB code 6H6P). Derivatisation of these crystals led to lower resolution diffraction and poor  
753 anomalous signal. These crystals were used for seeding which led to more consistent crystal shape  
754 however after these conditions had been left for over a month a more cubic shaped crystal form  
755 appeared in the condition. This new crystal form diffracted to 1.7 Å (PDB code 6H6O) and when used  
756 for seeding resulted in cubic crystal growth at a much lower protein concentration of 2.5 mg/ml. These

757 crystals natively diffracted well and were used for derivatisation using various heavy atom compounds.  
758 Polyvalan crystallophore No1 was used in cocrystallisation and resulted in crystals diffracting to 2 Å  
759 with significant anomalous signal allowing phasing of the data for structure determination (PDB code  
760 6H6N).

761 ***Data collection and structure determination:***

762 All crystals belong to the space group P212121. The 2.5 Å dataset has a unit cell parameters of a= 47.69  
763 Å; b= 94.55 Å; c= 115.66 Å, and four molecules per asymmetric unit. The 1.7 Å has a unit cell parameters  
764 of a= 48.6 Å; b= 67.87 Å; c= 74.60 Å, and the 2.05 Å dataset has a unit cell parameters of a= 48.30 Å;  
765 b= 67.93 Å; c= 73.08 Å, and both have two molecules per asymmetric unit. The 2.5 Å X-ray diffraction  
766 data were collected from single crystal at 100 K on the EIGER X 9M detector on beamline Proxima 2 at  
767 the Soleil synchrotron radiation source, Paris. The 1.7 Å and 2.05 Å X-ray diffraction data were collected  
768 from single crystals at 100 K on the Pilatus detector on beamline Proxima 1 at the Soleil synchrotron  
769 radiation source, Paris. Data were processed and scaled using the package XDS (Kabsch, 2010).  
770 Anisotropy and diffraction limits were set using the program STARANISO (Bricogne et al., 2017). Phases  
771 were obtained from 2 Terbium sites in the 2.05 Å dataset using SHELX (Sheldrick, 2015). Model was  
772 automatically built using Buccaneer and further manual building using the program Coot (Cowtan,  
773 2006; Emsley et al., 2010) with refinement performed using BUSTER (Bricogne et al., 2017). This model  
774 was then used to molecular replace the two other datasets for their further modelling and refinement  
775 using the same methods. The CCP4 software suite was also used in processing of data and Pymol was  
776 used in production of images including the CAVER3 plugin for cavities (Chovancova et al., 2012; Pavelka  
777 et al., 2016; Winn et al., 2011).

778 ***<sup>13</sup>C<sub>6</sub>-UQ<sub>8</sub> biosynthesis activity assay:***

779 *ΔubiC* cells from an overnight preculture were inoculated at OD<sub>600</sub> 0.15 in 100 mL MSG medium. The  
780 cells were cultured at 37°C, 200 rpm until OD<sub>600</sub> 1, at which point chloramphenicol (200 µg/mL) was

781 added and incubation continued for 20 min. Then, the cells were transferred to a screw cap flask fitted  
782 with two plastic tubing through which the culture was purge with argon for 5 min. 10  $\mu\text{M}$   $^{13}\text{C}_7$ -4-HB  
783 was added, the cells were maintained under argon at 37°C and initial experiments following the  
784 biosynthesis of  $^{13}\text{C}_6$ -OPP at different incubation time established that 10 min labeling with  $^{13}\text{C}_7$ -4-HB  
785 was optimal (Figure S1B). Cells were cooled down on ice under argon. In all subsequent steps carried  
786 out under normal atmosphere, care was taken to maintain the cells at 4°C in order to avoid conversion  
787 of  $^{13}\text{C}_6$ -OPP into  $^{13}\text{C}_6$ -UQ<sub>8</sub>. Cold cells were collected by centrifugation at 3,200 g, 4°C, 10 min and  
788 resuspended in 10 mL cold standard buffer (50 mM Tricine pH 7.5, 5 mM MgSO<sub>4</sub>, 10 mM  $\beta$ -  
789 mercaptoethanol).

790 For the *in vivo* assay, the cells were diluted five-fold in MSG medium containing 200  $\mu\text{g}/\text{mL}$   
791 chloramphenicol and incubated at 37°C either in normal atmosphere with 180 rpm shaking or under  
792 argon, a condition in which  $^{13}\text{C}_6$ -UQ<sub>8</sub> did not form. For the *in vitro* assay, cells in standard buffer were  
793 ruptured by 3 passages through French press (1500 psi) and debris were eliminated by centrifugation  
794 at 8,000 g for 20 min at 4°C. The cell free lysate was either directly assayed by adding 2 mM NADH, 2  
795 mM SAM and incubating at 30°C with occasional shaking or ultracentrifuged at 150,000 g, 4°C for 90  
796 min. The supernatant corresponding to the soluble fraction was taken and the membrane pellet was  
797 resuspended in a volume of standard buffer equal to that of the supernatant. 300  $\mu\text{L}$  fractions were  
798 assayed by adding 2 mM NADH and 2 mM SAM and incubating at 30°C with occasional shaking. The  
799 reactions were stopped by placing the samples at -20°C prior to lipid extraction and HPLC-ECD-MS  
800 analysis.

#### 801 ***Lipid extraction and quinone analysis:***

802 Quinone extraction from cell pellets and quantification by HPLC-ECD-MS analyses were performed as  
803 previously described (Loiseau et al., 2017). Aliquots of glycerol gradient fractions (250  $\mu\text{L}$ ) or of  
804 membrane (100  $\mu\text{L}$ ) and soluble fractions (400  $\mu\text{L}$ ) were extracted twice in glass tubes with 3 mL  
805 methanol, 30  $\mu\text{L}$  KCl 3M and 2\*2 mL p-ether. Depending on the separation needed, mobile phase 1

806 (40% ethanol, 40% acetonitrile and 20% of a mix of 90% isopropanol, 10% ammonium acetate (1 M),  
807 0.1% TFA) or mobile phase 2 (50% methanol, 40% ethanol and 10% of a mix of 90% isopropanol, 10%  
808 ammonium acetate (1 M), 0.1% TFA) were used at a flow rate of 1 mL/min with a BetaBasic-18 column.  
809 MS detection was on a MSQ spectrometer (Thermo Scientific) and electrospray ionization in positive  
810 mode used a probe temperature of 400°C and a cone voltage of 80V. Single ion monitoring (SIM)  
811 detected the following compounds: OPP (M+NH<sub>4</sub><sup>+</sup>), m/z 656.0-656.8, 5-10 min, scan time 0.2 s; <sup>13</sup>C<sub>6</sub>-  
812 OPP (M+ NH<sub>4</sub><sup>+</sup>), m/z 662.0-662.8, 5-10 min, scan time 0.2 s; DMQ<sub>8</sub> (M+NH<sub>4</sub><sup>+</sup>), m/z 714.0-714.8, 6-10  
813 min, scan time 0.2 s; UQ<sub>8</sub> (M+NH<sub>4</sub><sup>+</sup>), m/z 744.0-744.8, 6-10 min, scan time 0.2 s; <sup>13</sup>C<sub>6</sub>-UQ<sub>8</sub> (M+Na<sup>+</sup>), m/z  
814 755.0-755.8, 6-10 min, scan time 0.3 s; UQ<sub>10</sub> (M+NH<sub>4</sub><sup>+</sup>), m/z 880.2-881.2, 10-17 min, scan time 0.2 s.  
815 For some analyses, 0.1mM methylamine was added to the methanol of mobile phase 2, in which case  
816 the probe temperature was 450°C and SIMs were set as follows: UQ<sub>8</sub> (M+CH<sub>3</sub>NH<sub>3</sub><sup>+</sup>), m/z 758.0-758.8,  
817 6-10 min, scan time 0.2 s; <sup>13</sup>C<sub>6</sub>-UQ<sub>8</sub> (M+CH<sub>3</sub>NH<sub>3</sub><sup>+</sup>), m/z 764.0-764.8, 6-10 min, scan time 0.3 s; UQ<sub>10</sub>  
818 (M+CH<sub>3</sub>NH<sub>3</sub><sup>+</sup>), m/z 894.2-895.2, 10-17 min, scan time 0.2 s. MS spectra were recorded between m/z  
819 600 and 900 with a scan time of 0.3 s. UV detection at 247 nm was used to quantify DMK<sub>8</sub> and MK<sub>8</sub>.  
820 Peak areas were corrected for sample loss during extraction on the basis of the recovery of the UQ<sub>10</sub>  
821 internal standard and were then normalized either to cell's wet weight, or to total proteins (soluble  
822 and membrane fractions), or to quantity of purified protein, or to the total content of the fraction  
823 deposited on glycerol gradients. The peak of UQ<sub>8</sub> obtained with electrochemical detection was  
824 quantified with a standard curve of UQ<sub>10</sub> (Hajj Chehade et al., 2013).

#### 825 ***Purification of the Ubi complex:***

826 Ubi-SPA and WT strains were cultured in 1 L LB medium (2 L flasks) with shaking (200 rpm) at 37°C until  
827 OD<sub>600</sub> = 2.5-3. Soluble fractions were prepared in 10 mL lysis buffer (10 mM Tris-HCl pH 7.9, 100 mM  
828 NaCl, 10 mM DTT) with 1X anti-protease (sigmaFAST protease inhibitor). The subsequent two steps  
829 purification procedure was carried out at 4°C with ice cold buffers and we indicate below the names  
830 of the fractions analyzed in Figure S2c-g. 100 µL of anti-Flag M2 agarose beads (Sigma) were first  
831 washed with 10 mL lysis buffer (without DTT) and the beads were added to the ultracentrifugation

832 supernatant in a 15 mL Falcon tube. The mixture was rotated moderately (20 rpm) for 3h, then  
833 transferred to a 10 mL disposable chromatography column (EconoPac, BioRad) and the unbound  
834 material was eluted by gravity (FT-FLAG). The resin was washed with 30 mL lysis buffer (W1) followed  
835 by 30 mL TEV cleavage buffer (50 mM Tris-HCl pH 7.9, 100 mM NaCl and 0.2 mM EDTA) (W2). The 3X  
836 FLAG motif was cleaved by adding 500  $\mu$ L of TEV cleavage buffer containing 1X anti-protease, 1 mM  
837 DTT and 50  $\mu$ g Tobacco Etch Virus protease (TEV) and by incubating overnight under rotation. The next  
838 day, the eluate was drained into a fresh Eppendorf tube (EI-TEV).

839 Prior to use, 100  $\mu$ L of Calmodulin-Sepharose 4B beads were transferred into a 15 mL tube (Falcon),  
840 washed twice in batch with 10 mL of CBP binding buffer (10 mM Tris-HCl pH 7.9, 100 mM NaCl and 2  
841 mM  $\text{CaCl}_2$ ) and the beads were suspended in 500  $\mu$ L of TEV cleavage buffer containing 2 mM  $\text{CaCl}_2$  and  
842 10 mM  $\beta$ -mercaptoethanol. The beads suspension was added to the eluate recovered after TEV  
843 cleavage and the mixture was rotated for 3h in an Eppendorf tube. The sample was transferred to a  
844 1.5 mL chromatography column (Mini-columns, Evergreen Scientific) and the unbound fraction was  
845 drained by gravity (FT-CBP). The beads were then washed 5 times with 500  $\mu$ L of calmodulin washing  
846 buffer (10 mM Tris-HCl pH 7.9, 100 mM NaCl, 1 mM  $\text{CaCl}_2$ , 10 mM  $\beta$ -mercaptoethanol) (W1-W5). The  
847 bound proteins were eluted in five fractions of 100  $\mu$ L using the calmodulin elution buffer (10 mM Tris-  
848 HCl pH 7.9, 200 mM NaCl, 3 mM EGTA, 10 mM  $\beta$ -mercaptoethanol) (E1-E5).

849 ***Glycerol gradient fractionation:***

850 1 mL soluble fractions of Ubi-SPA strains were deposited on 10 mL linear glycerol gradients prepared  
851 using two glycerol solutions (densities 1.025 and 1.110) in modified buffer A (50 mM Imidazole/HCl  
852 pH=7, 50 mM NaCl, 1 mM EDTA). Fractions of 0.5 mL were collected manually from the bottom of the  
853 tubes after centrifugation for 15h at 120,000 g and 4°C in a swinging bucket rotor (Beckman SW-41).  
854 15  $\mu$ L of each fraction were analyzed by SDS-PAGE, SPA-tagged proteins were immunodetected and  
855 the reproducibility of the gradients was verified by immunodetection of GAPDH. Quinone  
856 quantification in glycerol gradient fractions was measured from 200  $\mu$ L aliquots.



857 ***Mass spectrometry-based proteomic analyses:***

858 Proteins were in-gel digested using trypsin as described in (Barral et al., 2017). The resulting peptides  
859 were analyzed by online nano-liquid chromatography coupled to tandem mass spectrometry (Ultimate  
860 3000 nano RSLC and Q-Exactive HF, Thermo Scientific) using a 120-min gradient. Peptides were  
861 sampled on a 300  $\mu\text{m}$  x 5 mm PepMap C18 precolumn (Thermo Scientific) and separated on a 75  $\mu\text{m}$  x  
862 250 mm C18 column (Reprosil-Pur 120 C18-AQ, 1.9  $\mu\text{m}$ , Dr. Maisch). MS and MS/MS data were  
863 acquired using Xcalibur (Thermo Scientific). Peptides and proteins were identified using Mascot  
864 (version 2.6.0) through concomitant searches against Uniprot (Escherichia coli K12 taxonomy), classical  
865 contaminants database (homemade) and the corresponding reversed databases. The Proline software  
866 (<http://proline.profi-proteomics.fr>) was used to filter the results (conservation of rank 1 peptides,  
867 peptide identification FDR < 1% as calculated on peptide scores by employing the reverse database  
868 strategy, minimum peptide score of 25, and minimum of 1 specific peptide per identified protein  
869 group) before performing a compilation, grouping and comparison of the protein groups from the  
870 different samples. Proteins were considered as potential partners of the bait if they were identified  
871 only in the positive co-IPs with a minimum of 5 specific spectral counts or enriched at least 10 times in  
872 positive co-IPs compared to control ones on the basis of specific spectral counts.

873

874 **QUANTIFICATION AND STATISTICAL ANALYSIS**

875 See each individual method and figure legends for the associated statistical analysis. In general, p-  
876 values were calculated using an unpaired, two-tailed, Student's t-test. In all cases, n represents  
877 independent replicates of an experiment.

878

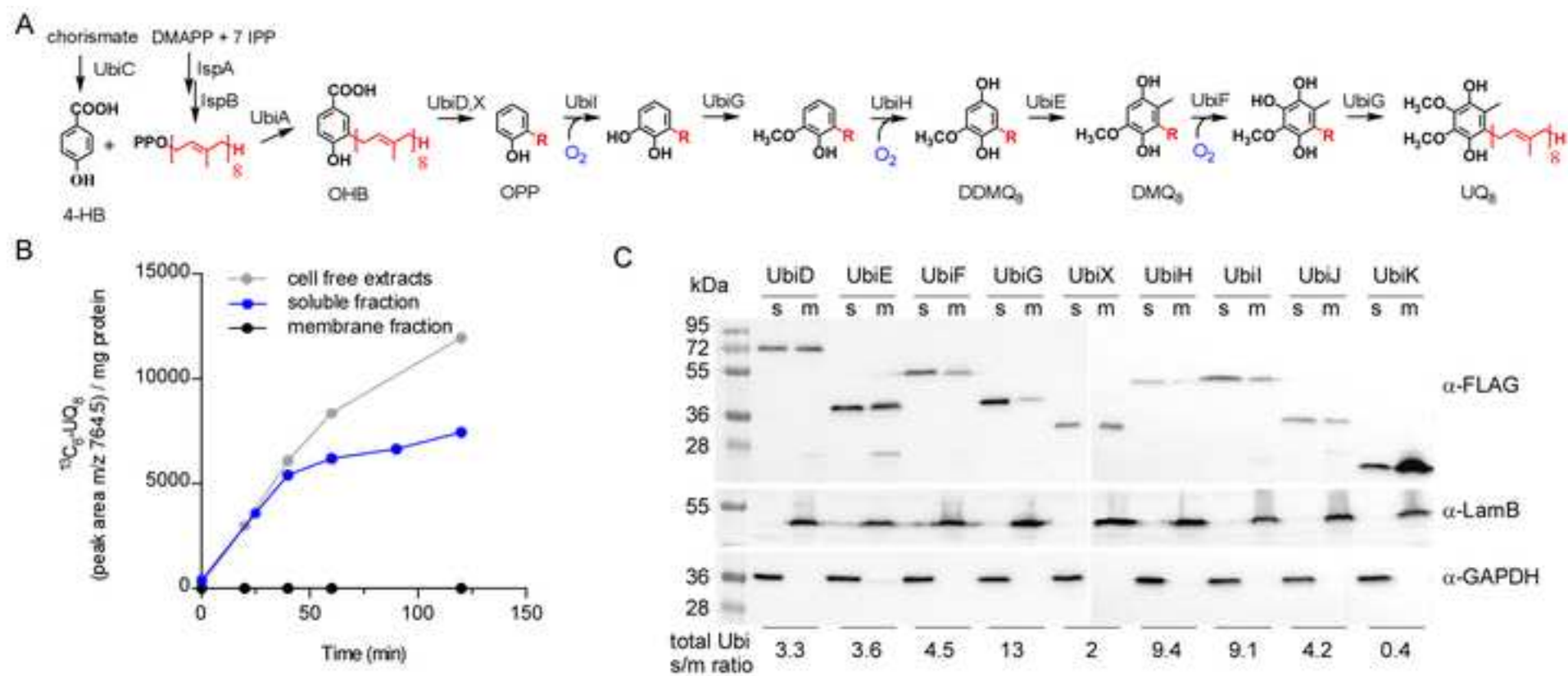
879 **DATA AND SOFTWARE AVAILABILITY**

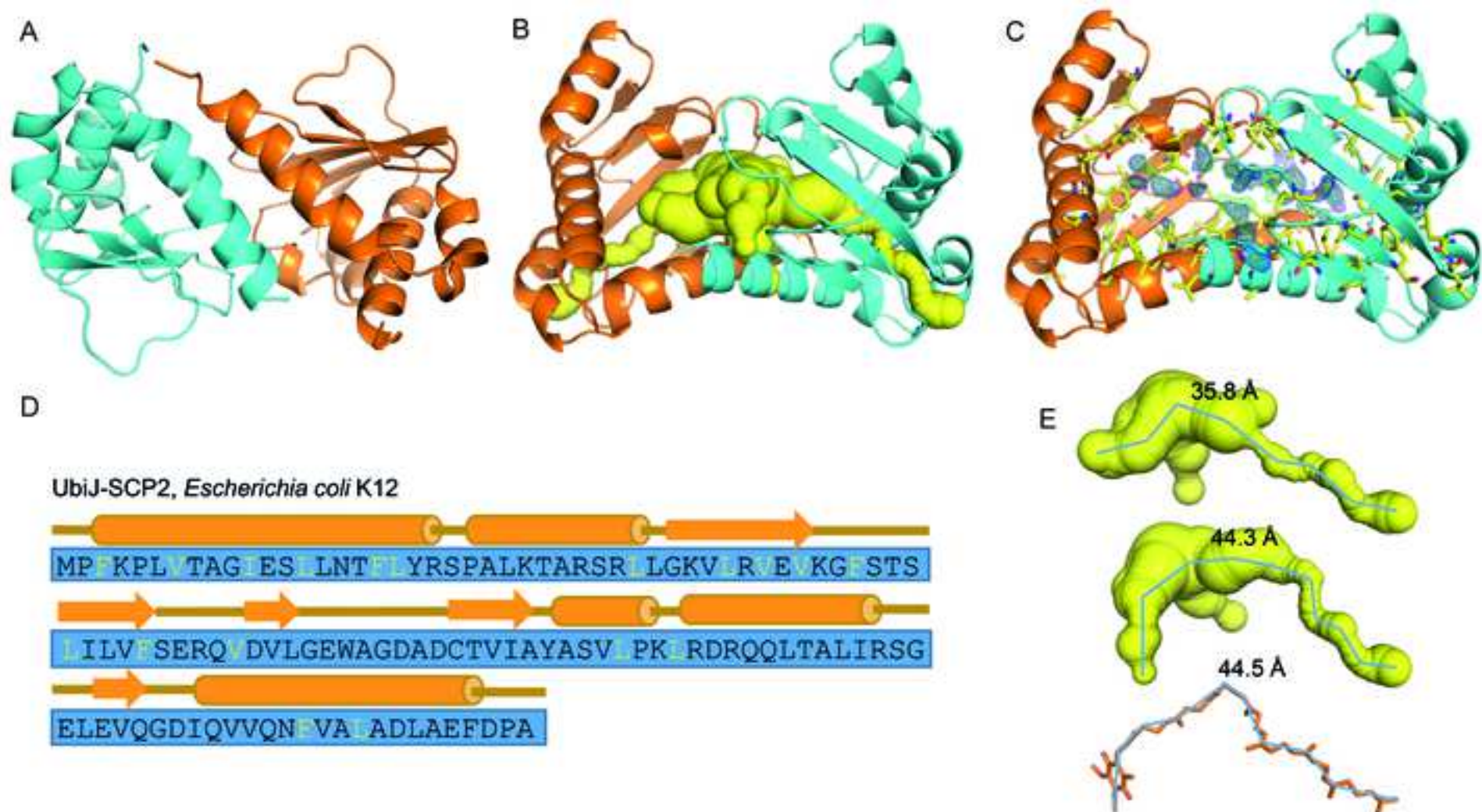
880 The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium  
881 via the PRIDE partner repository with the dataset identifier PXD010396.

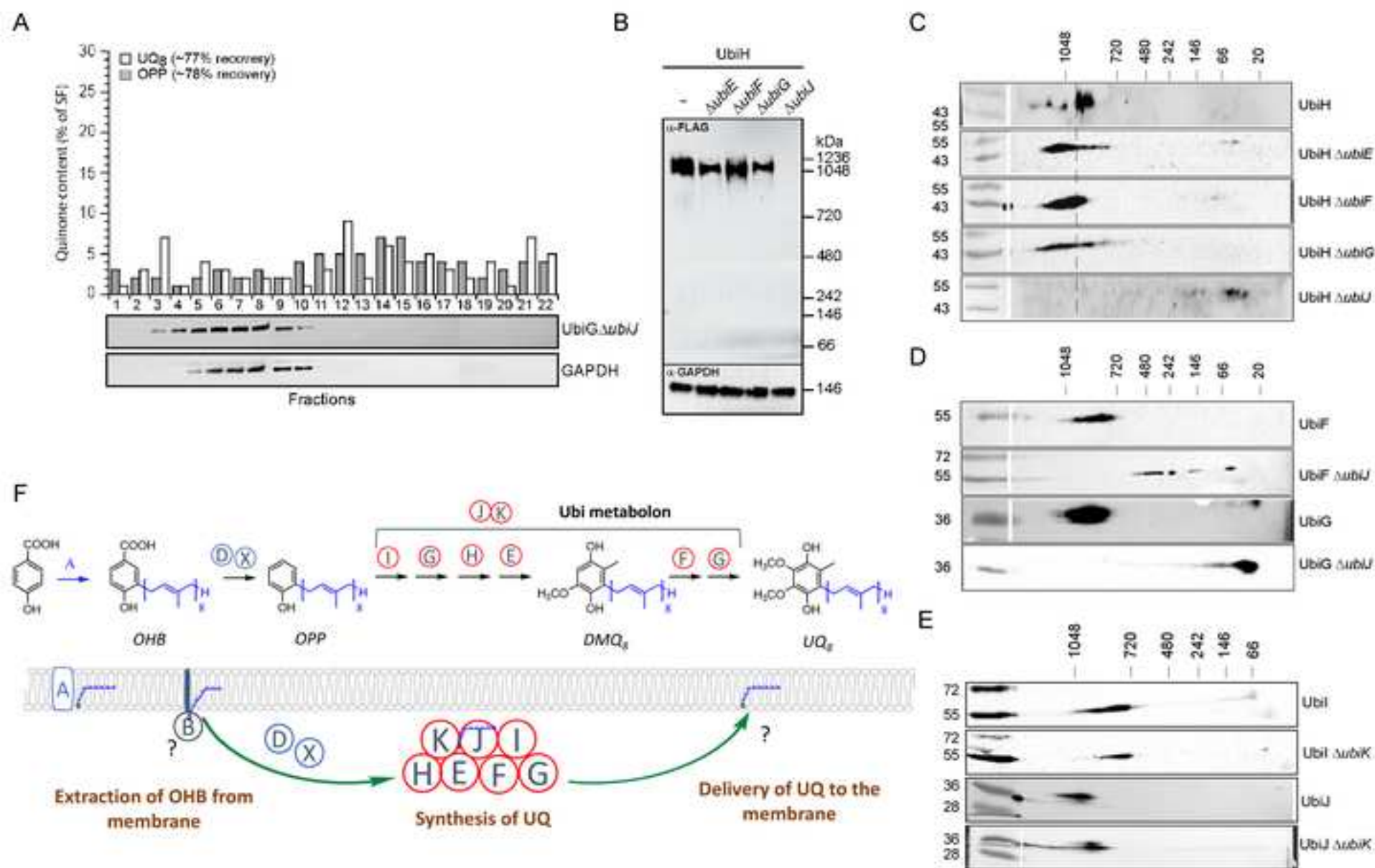
882 The crystal structures of the SCP2 domain of UbiJ have been deposited in the Protein Data Bank PDB  
883 with the identifiers 6H6N, 6H6O and 6H6P.

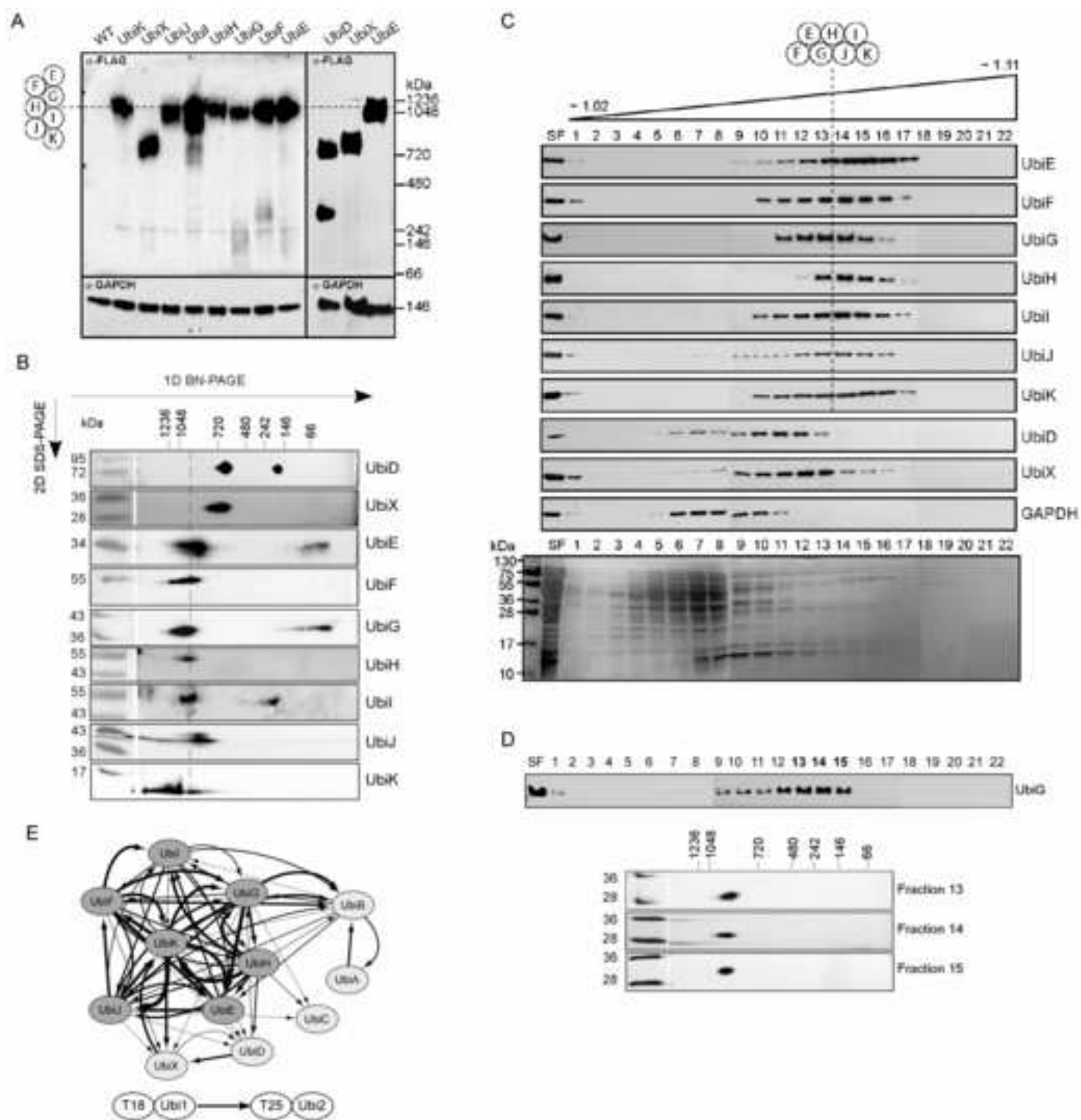
884 **Supplemental Information**

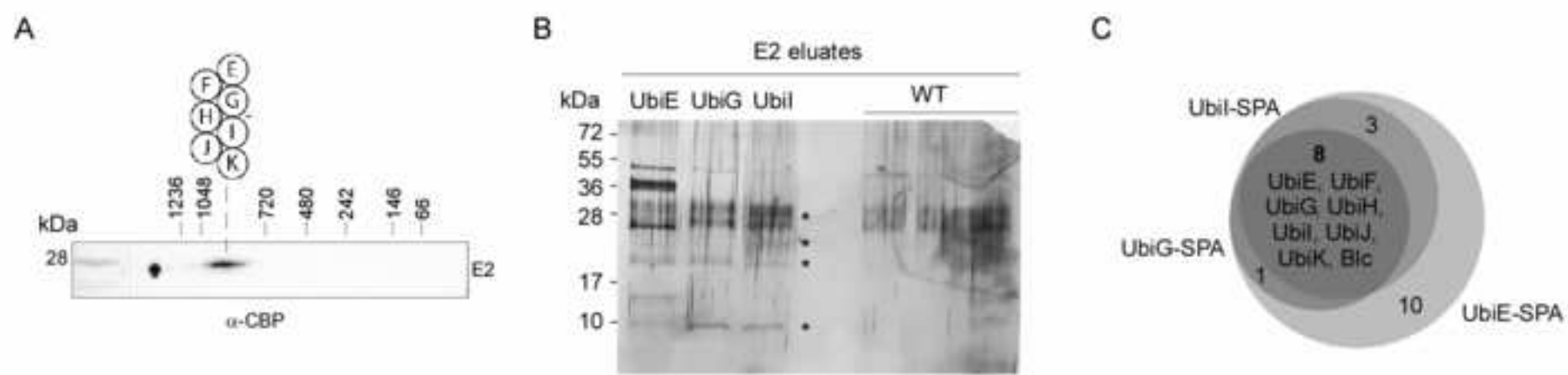
885 Supplemental information contains 6 supplemental figures and 6 supplemental tables.

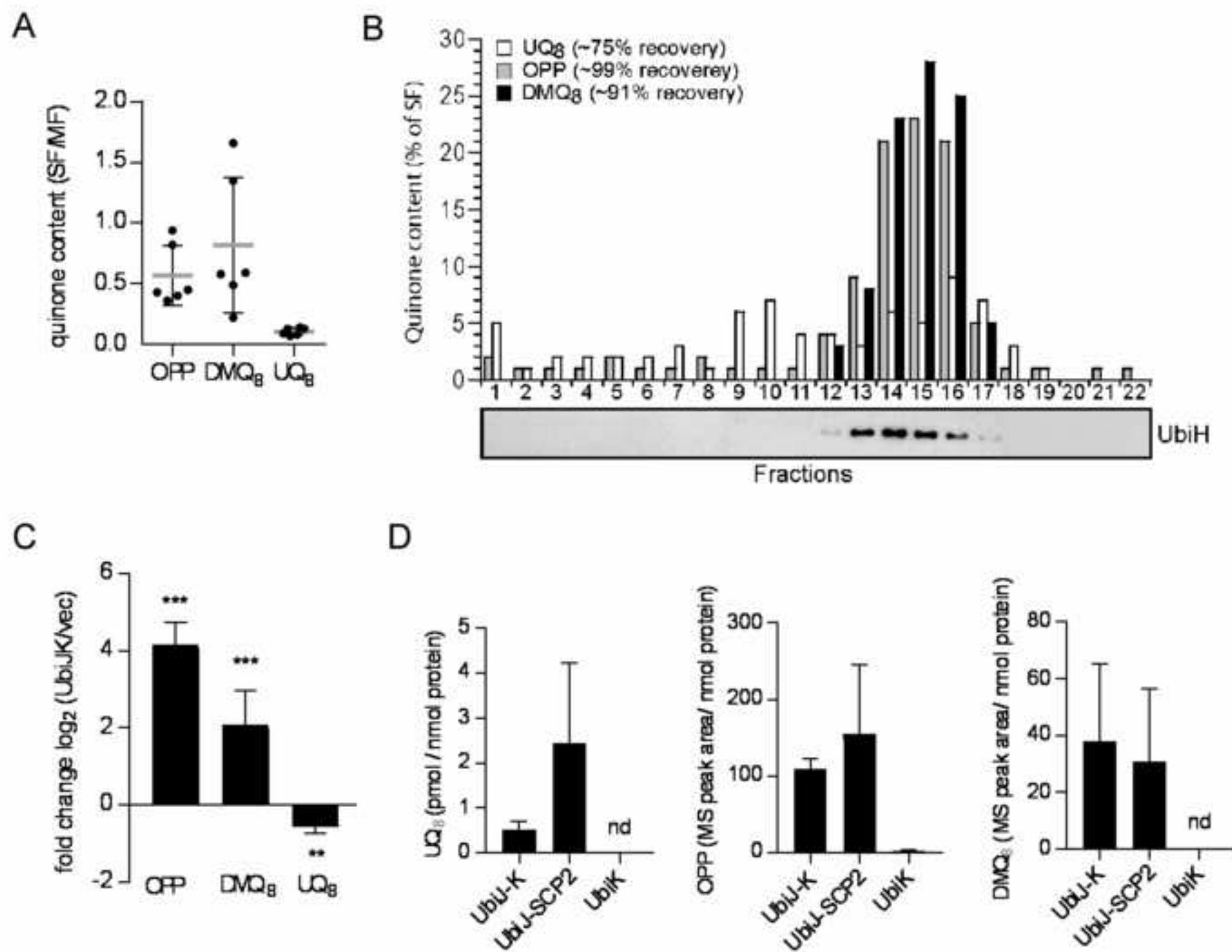














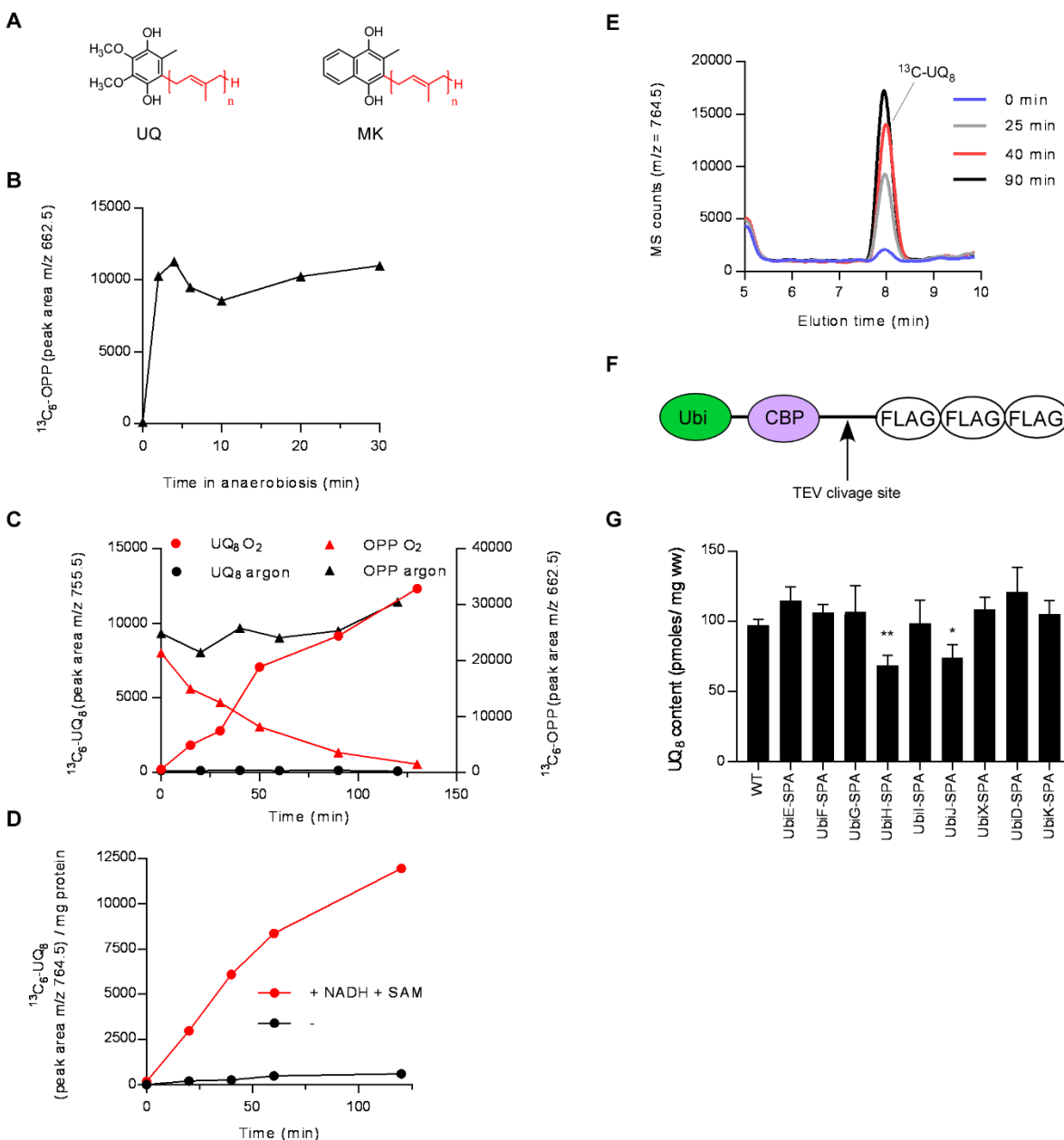
## KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
$\alpha$ -FLAG	Sigma Aldrich	Cat# A2220 (RRID:AB_10063035)
$\alpha$ -Calmodulin Binding Peptide	Genscript	Cat# A00635-40 (RRID:AB_914284)
$\alpha$ -GAPDH	Covalab	Cat# 00006357
$\alpha$ -LamB	A. Meinke, Intercell AG	N/A
goat anti-rabbit HRP-conjugated	Covalab	Cat# 00030130
goat anti-mouse HRP-conjugated	Covalab	Cat# 00030133
<b>Bacterial and Virus Strains</b>		
<i>E. coli</i> MG1655	ATCC	ATCC 47076
<i>E. coli</i> BW25113	National BioResource Project	<a href="http://nbrp.jp/">http://nbrp.jp/</a> search for ME9062
<i>E. coli</i> BL21 (DE3)	Merck Millipore	Cat# 69450-3
<i>E. coli</i> BTH101	(Karimova et al., 1998)	N/A
<i>E. coli</i> DY330	(Hu et al., 2009)	N/A
<b>Biological Samples</b>		
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
$^{13}\text{C}_7$ -4-HB	Sigma Aldrich	Cat# 587869
NADH	Sigma Aldrich	Cat# 43420
S-Adenosylmethionine	Sigma Aldrich	Cat# A2408
anti-Flag M2 agarose beads	Sigma Aldrich	Cat# A2220
Calmodulin-Sepharose 4B beads	Sigma Aldrich	Cat# GE17-0529-01
sigmaFAST protease inhibitor	Sigma Aldrich	Cat# S8830
Prestained (Two Colors) Protein Ladder Plus (10-250Kda)	Euromedex	Cat# 06P-0211
Native PAGE sample additive	Life Technologies	Cat# BN2003
Native PAGE 3–12% Bis–Tris gels	Life Technologies	Cat# BN1001BOX
Native PAGE 20X Running Buffer	Life Technologies	Cat# BN2001
NativeMark protein ladder	Life Technologies	Cat# LC0725
Polyvalan crystallophore No1	Molecular Dimensions	Cat# MD2-81
Purified UbiK protein	This study	N/A
Purified UbiJ-SCP2 protein	This study	N/A
Purified UbiJ-K complex	This study	N/A
Sequencing Grade Modified Trypsin	Promega	V5111
Dithiothreitol	Fisher Scientific	BP172
Iodoacetamide	Sigma Aldrich	I1149
Ammonium bicarbonate	Sigma Aldrich	A6141
Formic acid	EMD Millipore Chemical	1.11670.0250
Acetonitrile	Sigma Aldrich	34851-M

Critical Commercial Assays		
Deposited Data		
mass spectrometry proteomics data	This study	<a href="http://www.proteomeexchange.org">http://www.proteomeexchange.org</a> PXD010396
X-ray structure of UbiJ-SCP2, 2.1 Å	This study	<a href="http://www.rcsb.org/">http://www.rcsb.org/</a> PDB ID: 6H6N
X-ray structure of UbiJ-SCP2, 1.7 Å	This study	PDB ID: 6H6O
X-ray structure of UbiJ-SCP2, 2.5 Å	This study	PDB ID: 6H6P
Experimental Models: Cell Lines		
Experimental Models: Organisms/Strains		
For all <i>E. coli</i> strains, see Table S5		
Oligonucleotides		
For all primers, see Table S6	This paper	N/A
Recombinant DNA		
pETDuet-6his-ubiJ/ubiK	(Loiseau et al., 2017)	N/A
pETDuet-ubiJ( $\Delta$ 121–201)	(Loiseau et al., 2017)	N/A
pETDuet-ubiK no-tag	(Loiseau et al., 2017)	N/A
pT18	(Karimova et al., 1998)	N/A
pT25	(Karimova et al., 1998)	N/A
pT18-ubiA	This paper	N/A
pT18-ubiB	This paper	N/A
pT18-ubiC	This paper	N/A
pT18-ubiD	This paper	N/A
pT18-ubiE	This paper	N/A
pT18-ubiF	This paper	N/A
pT18-ubiG	This paper	N/A
pT18-ubiH	This paper	N/A
pT18-ubil	This paper	N/A
pT18-ubiJ	This paper	N/A
pT18-ubiK	This paper	N/A
pT18-ubiX	This paper	N/A
pT25-ubiA	This paper	N/A
pT25-ubiB	This paper	N/A
pT25-ubiC	This paper	N/A
pT25-ubiD	This paper	N/A
pT25-ubiE	This paper	N/A
pT25-ubiF	This paper	N/A
pT25-ubiG	This paper	N/A
pT25-ubiH	This paper	N/A
pT25-ubil	This paper	N/A
pT25-ubiJ	This paper	N/A
pT25-ubiK	This paper	N/A

pT25-ubiX	This paper	N/A
Software and Algorithms		
XDS	(Kabsch, 2010)	<a href="http://xds.mpimf-heidelberg.mpg.de/">http://xds.mpimf-heidelberg.mpg.de/</a>
STARANISO	(Bricogne et al., 2017)	<a href="http://staraniso.globalphasing.org/cgi-bin/staraniso.cgi">http://staraniso.globalphasing.org/cgi-bin/staraniso.cgi</a>
SHELXL	(Sheldrick, 2015)	<a href="http://shelx.uni-goettingen.de/">http://shelx.uni-goettingen.de/</a>
Coot	(Emsley et al, 2010)	<a href="https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/">https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/</a>
BUSTER	(Bricogne et al., 2017)	<a href="https://www.globalphasing.com/buster/">https://www.globalphasing.com/buster/</a>
CCP4	(Winn et al., 2011)	<a href="http://www.ccp4.ac.uk/">http://www.ccp4.ac.uk/</a>
Pymol	Schrödinger, LLC	<a href="https://pymol.org/2/">https://pymol.org/2/</a>
CAVER3	(Chovancova et al., 2012)	<a href="https://www.caver.cz/">https://www.caver.cz/</a>
Mascot Distiller	Matrix Science	<a href="http://www.matrixscience.com/">http://www.matrixscience.com/</a>
Mascot	Matrix Science	<a href="http://www.matrixscience.com/">http://www.matrixscience.com/</a>
Proline	Open source	<a href="http://www.profiptroteomics.fr/proline">http://www.profiptroteomics.fr/proline</a>
Xcalibur	Thermo Fisher Scientific	<a href="https://www.thermofisher.com">https://www.thermofisher.com</a>
Cytoscape 3.0	(Su et al., 2014)	<a href="https://cytoscape.org">https://cytoscape.org</a>
Other		
Liquid chromatography	Thermo Scientific	UltiMate 3000 RSLCnano System
Reprosil-Pur 120 C18-AQ, 1.9 $\mu\text{m}$ , 250 mm x 75 $\mu\text{m}$	Dr. Maisch GmbH	r119.aq.
Mass spectrometer	Thermo Scientific	Q Exactive Plus
BetaBasic-18, 150 mm x 4.6 mm, 5 $\mu\text{m}$	Thermo Scientific	Cat# 71505-154630
Hitrap Chelating HP column	GE Healthcare	Cat# GE17-0408-01

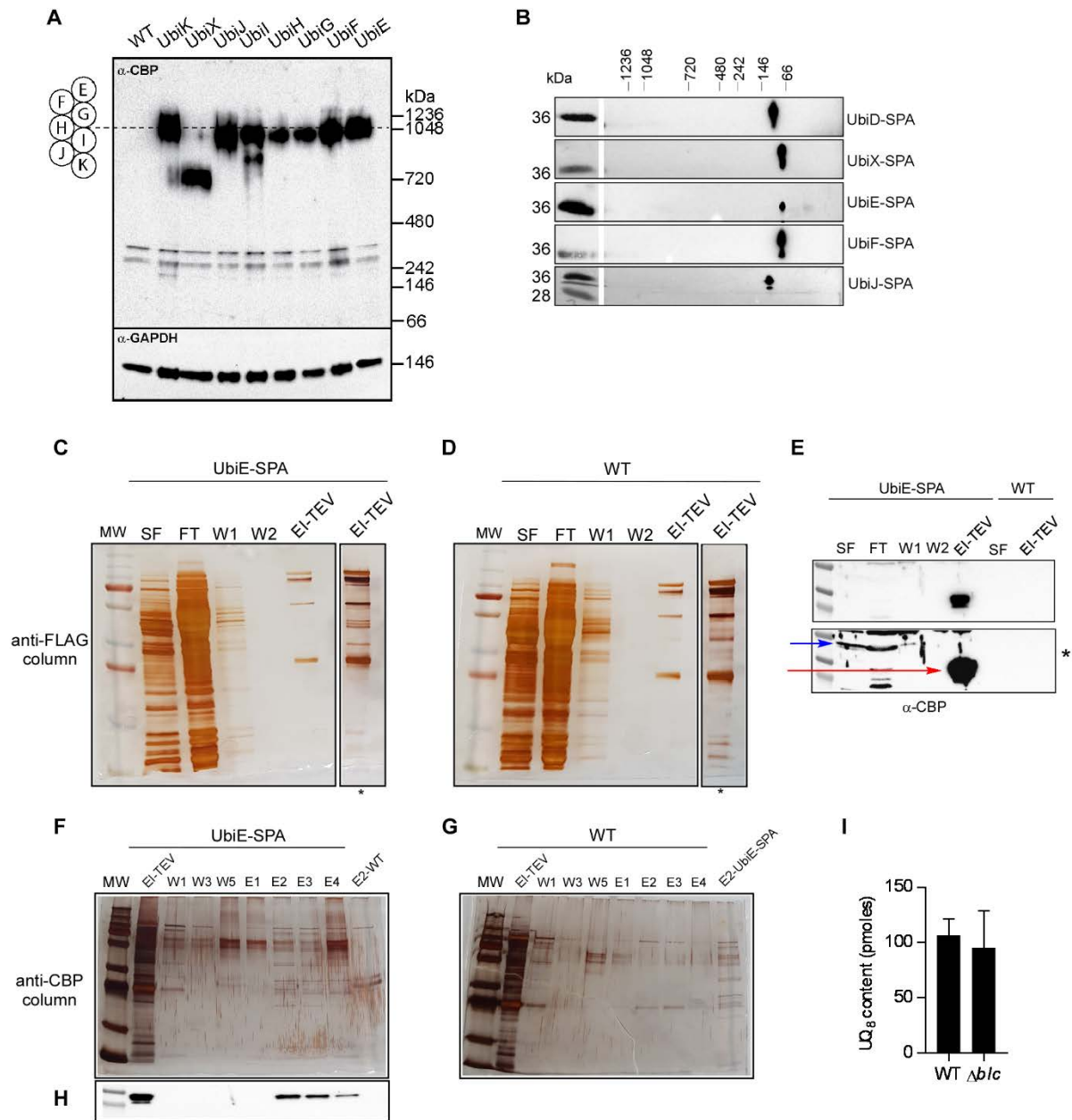
## FIGURE S1



**Figure S1 related to figure 1:** A) Structures of Ubiquinone (UQ) and menaquinone (MK) with the head groups in black. The length of the polyisoprenyl tail (in red) can vary between species ( $n=6-10$ ). B) Accumulation of  $^{13}\text{C}_6$ -OPP ( $\text{M}^+ \text{NH}_4^+$ ) after addition of  $^{13}\text{C}_7$ -4-HB to  $\Delta\text{ubiC}$  cells incubated in anaerobic conditions. C) Conversion of  $^{13}\text{C}_6$ -OPP ( $\text{M}^+ \text{NH}_4^+$ ) into  $^{13}\text{C}_6$ -UQ<sub>8</sub> ( $\text{M}^+ \text{Na}^+$ ) by cells incubated in air or maintained under argon. D) Synthesis of  $^{13}\text{C}_6$ -UQ<sub>8</sub> ( $\text{M}^+ \text{CH}_3\text{NH}_3^+$ ) by cell free extracts incubated in air with or without addition of NADH and SAM. E) HPLC analysis and single ion monitoring ( $m/z=764.5$ ,  $\text{M}^+ \text{CH}_3\text{NH}_3^+$ ) of  $^{13}\text{C}_6$ -UQ<sub>8</sub> in cytoplasmic fractions from  $\Delta\text{ubiC}$  cells preloaded with  $^{13}\text{C}$ -OPP and incubated in air for the

1  
2  
3  
4 indicated time in the presence of NADH and SAM. Results shown are representative of two (B, C) or three  
5 independent experiments (D, E). F) Schematic representation of a Ubi protein fused to the SPA tag, a  
6 repetition of 3 FLAG sequences separated from a CBP (Calmodulin Binding Protein) domain by a TEV clivage  
7 site (TEV: Tobacco Etch Virus). G) HPLC-ECD quantification of cellular UQ<sub>8</sub> content of *E. coli* strains carrying  
8 Ubi proteins endogenously tagged with SPA (mean ±SEM, n=3, \*: p< 0.05, \*\*: p< 0.01, unpaired Student's  
9 t test). The UQ<sub>8</sub> decrease in UbiH-SPA and UbiJ-SPA cells is statistically significant but nevertheless reflects  
10 that the UbiH-SPA and UbiJ-SPA proteins remain partially functional since a complete inactivation would  
11 cause a lack of UQ<sub>8</sub>, as observed in *ubiH* and *ubiJ* knock-out cells.  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

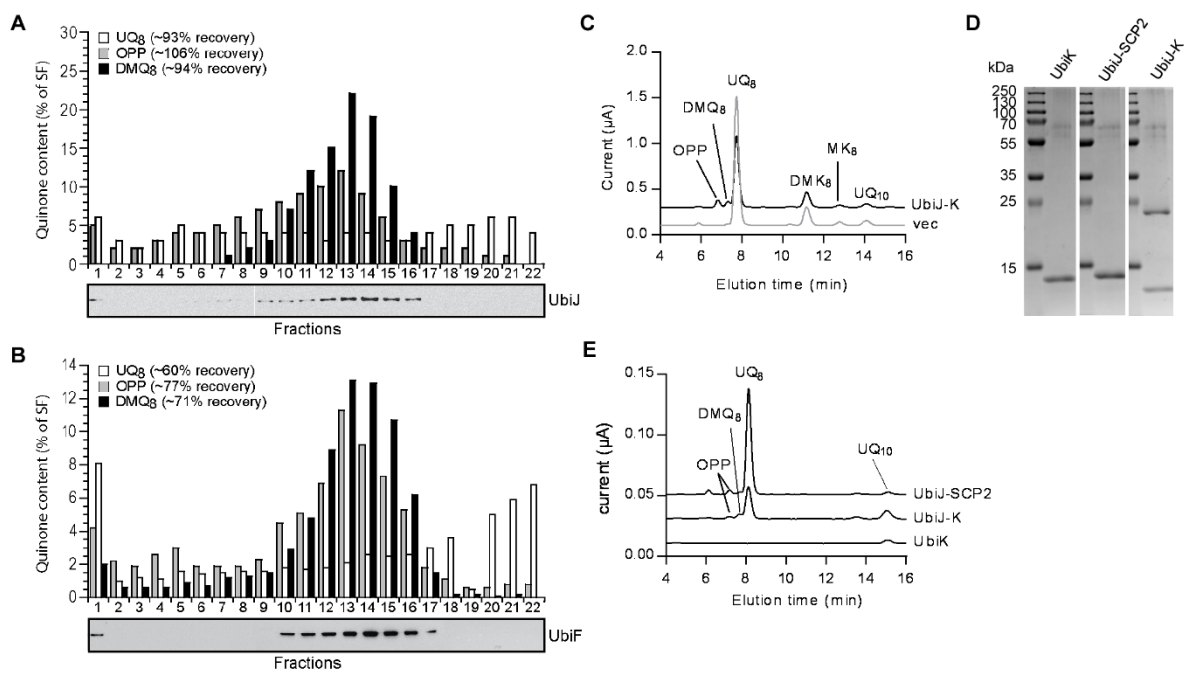
Figure S2



**Figure S2 related to figure 2 and 3:** A) BN-PAGE analysis and immunodetection of soluble fractions from the indicated Ubi-SPA strains (50  $\mu$ g of total proteins). The membrane was first probed with  $\alpha$ -CBP antibody, stripped and probed with  $\alpha$ -GAPDH (loading control). B) Immunodetection of the control GAPDH protein after stripping the  $\alpha$ -FLAG antibody from the membranes presented in Figure 2B. C-D) 20  $\mu$ L aliquots of soluble fraction (SF), flow-through (FT), washes 1 and 2 (W1, W2), and eluates after TEV digestion (EI-TEV) collected during the anti-FLAG purification step were analyzed by SDS-PAGE and the gel was colored by silver staining (extracts from the UbiE-SPA strain (C) and from the WT strain (D)). E)

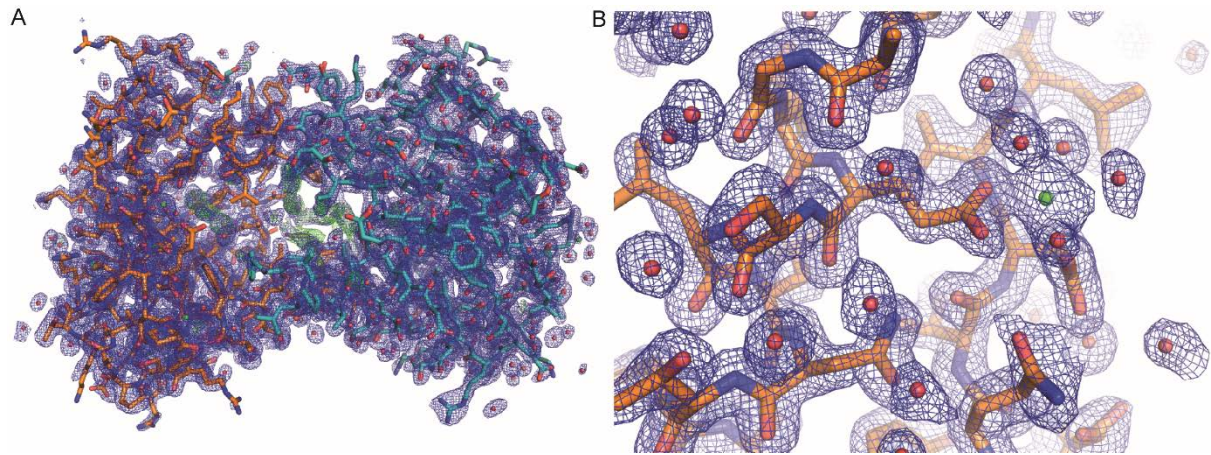
Western-blot analysis with  $\alpha$ -CBP antibody of samples described in C-D. Sizes of the UbiE-SPA protein before and after cleavage by TEV are indicated with blue and red arrows, respectively. Asterisks mark longer exposures (C-E). F-G) SDS-PAGE analysis and silver staining of fractions collected during the calmodulin purification step: washes 1, 3, 5 (W1, W3, W5) and eluates 1-4 (E1-E4) from purification with UbiE-SPA (F) and WT (G) samples. H) Western-blot analysis with  $\alpha$ -CBP antibody of samples described in F. I) UQ<sub>8</sub> quantification in  $\Delta b/c$  and WT strains (mean  $\pm$ SEM, n=4).

**Figure S3**



**Figure S3 related to figure 4:** A-B) Glycerol gradient analysis of soluble extracts from UbiJ-SPA and UbiF-SPA cells, and quantification of isoprenoid quinones in each fraction (see also Figure 4B). Western-blot are the same than in figure 2C. C) HPLC-ECD analyses (mobile phase 2) of lipid extracts from 1 mg of the *E. coli* cells containing an empty vector (vec) or overexpressing UbiJ(6His)-K and grown in LB medium (UQ<sub>10</sub> used as standard), DMK<sub>8</sub> for C2-demethylmenaquinone 8. D) SDS-PAGE analysis and coomassie blue staining of purified UbiK, UbiJ-K and UbiJ-SCP2. E) HPLC-ECD analyses (mobile phase 2) of lipids extracted from 4 nmoles of purified proteins (UQ<sub>10</sub> used as standard).

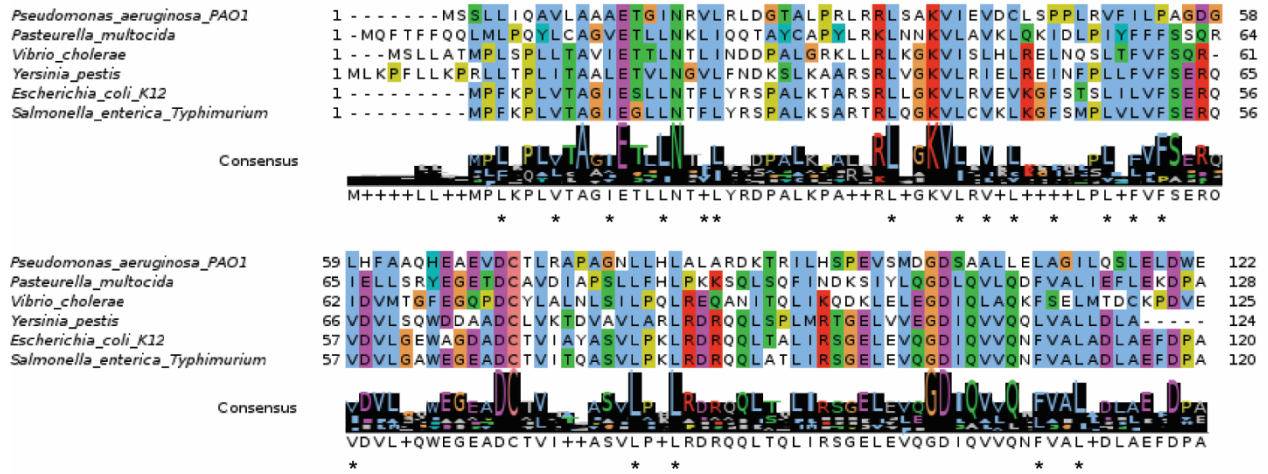
1  
2  
3  
4 **Figure S4**  
5  
6  
7  
8



**Figure S4 related to figure 5:** Stick model of the structure of UbiJ-SCP2 at 1.7 Å resolution with electron density maps. A) UbiJ-SCP2 dimer with maps for 2FoFc set at 1  $\sigma$  (blue mesh) and for FoFc set at 3  $\sigma$  (green mesh). B) Zoomed in region of chain A with maps for 2FoFc set at 1  $\sigma$ . (chain A shown in orange, chain B in teal, waters shown as red spheres, calcium shown as green spheres).

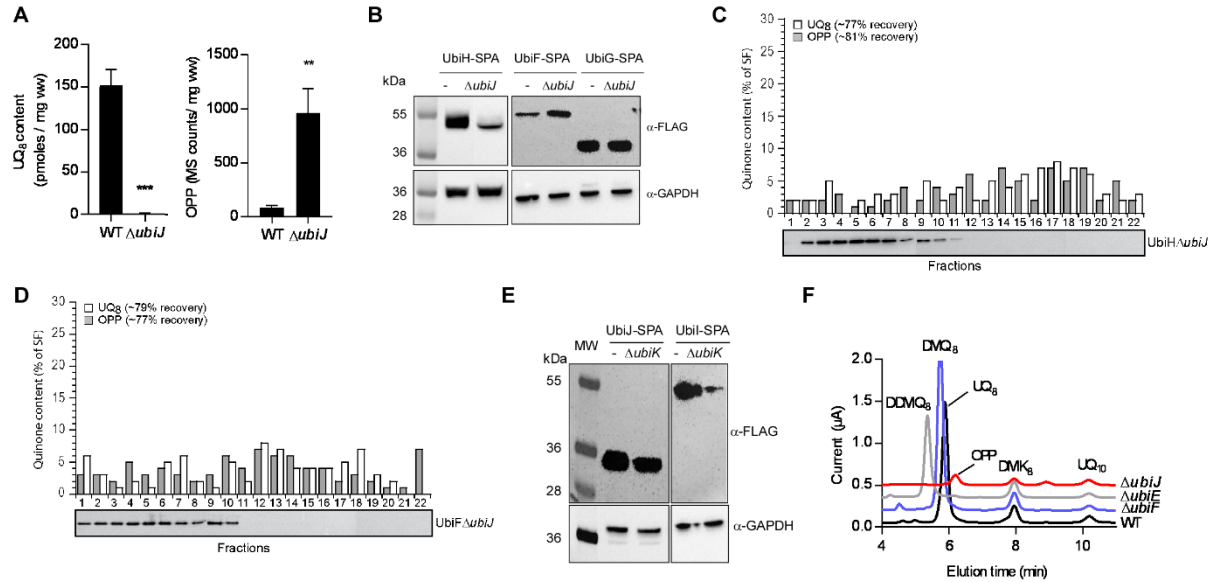


## Figure S5



**Figure S5 related to figure 5:** Sequence alignment of UbiJ-SCP2 domains taken from various bacterial strains. Residues lining the hydrophobic cavity highlighted in figure 5c are indicated by \*. NCBI sequence numbers: NP\_253751.1, WP\_005755273.1, NP\_229743.1, NP\_407227.1, NP\_418278.1, NP\_462856.1

## Figure S6



**Figure S6 related to figure 6:** A) UQ<sub>8</sub> and OPP content (normalized to cells wet weight) of WT and  $\Delta ubiJ$  cells grown in LB medium. B, E) Immunodetection of Ubi-SPA proteins ( $\alpha$ -FLAG antibody) in the soluble fractions of the indicated strains (25  $\mu$ g of proteins), with GAPDH as control. C-D) Glycerol gradient analysis of soluble extracts from UbiH-SPA  $\Delta ubiJ$  cells (C) or UbiF-SPA  $\Delta ubiJ$  cells (D) and quantification of OPP and UQ<sub>8</sub> in each fraction by HPLC-ECD-MS. Immunodetection with  $\alpha$ -FLAG antibody showing the position UbiH-SPA and UbiF-SPA in fractions of the glycerol gradient (see also Figure 6A). F) HPLC-ECD analyses (mobile phase 1) of lipid extracts from 1 mg of the indicated *E. coli* cells grown in LB medium (UQ<sub>10</sub> used as standard).

**Table S1 related to figure 2E:** Values of the bacterial two-hybrid assay, provided as separate excel file

**Table S2 related to figure 3:** Results of proteomic analysis, provided as separate excel file

**Table S3 related to figure 5:** Crystallography data table

Dataset	Terbium	Native Dimer	Native Tetramer
PDB code	6H6N	6H6O	6H6P
Space group	P 21 21 21	P 21 21 21	P 21 21 21
Unit cell dimensions			
a (Å)	48,3	48,66	47,69
b (Å)	67,93	67,87	94,55
c (Å)	73,08	74,61	115,66
Wavelength (Å)	1,6488	0,9786	0,9786
Resolution (Å)	40.29-2.11	39.55-1.70	47.28-2.5
Highest resolution bin (Å)	2.17-2.11	1.74-1.70	2.59-2.5
No. of reflections	307092	177660	137979
No. of unique reflections	13906	28017	18749
Completeness (%)	97.1 (60.7)	99.7 (96.7)	99.92 (99.94)
Rmerge	0.116 (0.709)	0.081 (1.957)	0.1602 (2.524)
I/σ (I)	12.3 (1.7)	10.7 (0.9)	9.13 (0.5)
CC1/2	0.998 (0.780)	0.999 (0.523)	0.998 (0.885)
Multiplicity	22.1 (9.3)	6.3 (6.1)	7.4 (7.7)
Refinement			
Rwork	0.194 (0.189)	0.202 (0.242)	0.233 (0.224)
Rfree	0.229 (0.227)	0.248 (0.271)	0.289 (0.258)
Average B-factor (Å <sup>2</sup> )			
Overall	43,05	32,65	79,12
From Wilson plot	32,35	22,83	72,21
No. of atoms			
Protein	1874	1851	3585
Water	118	185	89
RMS deviations from ideal			
Bond lengths (Å)	0,010	0,010	0,010
Bond angles (o)	1,07	0,98	1,27
Ramachandran outliers (%)			
Residues in most favored regions (%)	98,75	99,57	98,24
Residues in allowed regions (%)	1,25	0,43	1,76
Outliers (%)	0	0	0
*Values in parentheses are for the highest resolution bin.			

**Table S4** related to figure 5: Structurally characterized SCP2 proteins and their structural alignment with UbiJ-SCP2

Protein	Organism	Technique	Resolution	PDB	RMSD	lipid content	Sequence coverage	Sequence Identity
Alkyl sulfatase	<i>Pseudomonas</i> sp. DSM 6611	X-ray Diffraction	2,7	2YHE	1,563	-	67	20
Alkyl sulfatase	<i>Pseudomonas</i> sp. DSM 6611	X-ray Diffraction	3	4AV7	2,138	-	67	20
SCP2	Human	X-ray Diffraction	1,75	1IKT	2,162	Triton X-100	83	14
SCP2	<i>Archaeoglobus fulgidus</i>	X-ray Diffraction	2,11	3BN8	2,241	-	81	11
Alkyl sulfatase	<i>Pseudomonas</i> sp. S9	X-ray Diffraction	1,761	4NUR	2,474	-	67	17
SCP2	<i>Aedes aegypti</i>	X-ray Diffraction	2	3BDQ	2,591	palmitic acid	90	18
SCP2	<i>Helicoverpa armigera</i>	NMR	n/a	4UEI	2,619	-	74	12
SCP2	<i>Aedes aegypti</i>	X-ray Diffraction	1,7	2QZT	2,983	palmitic acid	90	18
SCP2	Yeast <i>Yarrowia Lipolytica</i>	X-ray Diffraction	2,2	4JGX	3,402	palmitic acid	79	14
SCP2	<i>Oryctolagus cuniculus</i>	X-ray Diffraction	1,8	1C44	3,483	-	68	18
SCP2	<i>Homo sapiens</i>	NMR	n/a	1QND	3,854	-	68	17
-	<i>Archaeoglobus fulgidus</i>	X-ray Diffraction	1,9	3CNU	4,084	-	90	11
SCP2	<i>Aedes aegypti</i>	NMR	n/a	2NBM	4,473	-	85	18

**Table S5** related to figure 1, 2: *E. coli* strains used in this study

strain	type	relevant genotype	source or reference
BW25113		<i>lacI</i> <sup>q</sup> <i>rrnB</i> <sub>T14</sub> <i>lacZ</i> <sub>WJ16</sub> <i>hsdR514</i> <i>araBAD</i> <i>rhaBAD</i> <sub>LD78</sub>	National BioResource Project
MG1655		Wild type	ATCC
BTH101		<i>F'</i> , <i>cya-99</i> , <i>araD139</i> , <i>galE15</i> , <i>galk16</i> , <i>rpsL1</i> ( <i>Str</i> <sup>R</sup> ), <i>hsdR2</i> , <i>mcrA1</i> , <i>mcrB1</i>	Karimova <i>et al</i> , 1998
DY330		W3110 $\Delta$ lacU169 gal490 $\lambda$ CI857 $\Delta$ (cro-bioA)	Hu <i>et al</i> , 2009
BL21 (DE3)		<i>fhuA2</i> [ <i>lon</i> ] <i>ompT</i> <i>gal</i> ( $\lambda$ DE3) [ <i>dcm</i> ] $\Delta$ <i>hsdS</i> $\lambda$ DE3	Merck Millipore
	<b>Keio strains</b>		<i>Keio</i> collection (Baba <i>et al</i> , 2006)
BW25113		<i>lacI</i> <sup>q</sup> <i>rrnB</i> <sub>T14</sub> <i>lacZ</i> <sub>WJ16</sub> <i>hsdR514</i> <i>araBAD</i> <i>rhaBAD</i> <sub>LD78</sub>	Laboratory collection
BW25113 $\Delta$ <i>ubiC</i>		As BW25113 but $\Delta$ <i>ubiC</i> ::Kan	JW5713
BW25113 $\Delta$ <i>ubiE</i>		As BW25113 but $\Delta$ <i>ubiE</i> ::Kan	JW5581
BW25113 $\Delta$ <i>ubiF</i>		As BW25113 but $\Delta$ <i>ubiF</i> ::Kan	JW0659
BW25113 $\Delta$ <i>ubiG</i>		As BW25113 but $\Delta$ <i>ubiG</i> ::Kan	JW2226
BW25113 $\Delta$ <i>blc</i>		As BW25113 but $\Delta$ <i>blc</i> ::Kan	JW4110
	<b>Knock-out strains</b>		
MG1655 $\Delta$ <i>ubiE</i>		As MG1655 but $\Delta$ <i>ubiE</i> ::Kan	Hajj Chehade <i>et al</i> , 2013
MG1655 $\Delta$ <i>ubiF</i>		As MG1655 but $\Delta$ <i>ubiF</i> ::Kan	Hajj Chehade <i>et al</i> , 2013
MG1655 $\Delta$ <i>ubiI</i>		As MG1655 but $\Delta$ <i>ubiI</i> ::Kan	Aussel <i>et al</i> , 2014
	<b>SPA-strains</b>		
DY330		W3110 $\Delta$ lacU169 gal490 $\lambda$ CI857 $\Delta$ (cro-bioA)	Hu <i>et al</i> , 2009
DY330 <i>ubiC</i> -SPA		As DY330 but <i>ubiC</i> -SPA::Kan	GI number 90111677
DY330 <i>ubiF</i> -SPA		As DY330 but <i>ubiF</i> -SPA::Kan	GI number 16128645
DY330 <i>ubiG</i> -SPA		As DY330 but <i>ubiG</i> -SPA::Kan	GI number 16130167
DY330 <i>ubiH</i> -SPA		As DY330 but <i>ubiH</i> -SPA::Kan	GI number 16130809
DY330 <i>ubiX</i> -SPA		As DY330 but <i>ubiX</i> -SPA::Kan	GI number 16130246
BW25113 <i>ubiD</i> -SPA		As BW25113 but <i>ubiD</i> -SPA::Kan	this work
BW25113 <i>ubiI</i> -SPA		As BW25113 but <i>ubiI</i> -SPA::Kan	this work
MG1655 <i>ubiC</i> -SPA		MG1655 +P1/DY330 <i>ubiC</i> -SPA::Kan	this work
MG1655 <i>ubiD</i> -SPA		MG1655 +P1/BW25113 <i>ubiD</i> -SPA::Kan	this work
MG1655 <i>ubiE</i> -SPA		<i>ubiE</i> -SPA::Kan	Loiseau <i>et al</i> , 2017
MG1655 <i>ubiF</i> -SPA		MG1655 +P1/DY330 <i>ubiF</i> -SPA::Kan	this work
MG1655 <i>ubiG</i> -SPA		MG1655 +P1/DY330 <i>ubiG</i> -SPA::Kan	this work
MG1655 <i>ubiH</i> -SPA		MG1655 +P1/DY330 <i>ubiH</i> -SPA::Kan	this work
MG1655 <i>ubiI</i> -SPA		MG1655 +P1/BW25113 <i>ubiI</i> -SPA::Kan	this work
MG1655 <i>ubiJ</i> -SPA		<i>ubiJ</i> -SPA::Kan	Loiseau <i>et al</i> , 2017
MG1655 <i>ubiK</i> -SPA		<i>ubiK</i> -SPA::Kan	Loiseau <i>et al</i> , 2017
MG1655 <i>ubiX</i> -SPA		MG1655 +P1/DY330 <i>ubiX</i> -SPA::Kan	this work
MG1655 <i>ubiH</i> -SPA $\Delta$ <i>ubiE</i>		$\Delta$ <i>ubiE</i> (cured with pCP20) + P1/ <i>ubiH</i> -SPA::Kan	this work
MG1655 <i>ubiH</i> -SPA $\Delta$ <i>ubiF</i>		$\Delta$ <i>ubiF</i> (cured with pCP20) + P1/ <i>ubiH</i> -SPA::Kan	this work
MG1655 <i>ubiH</i> -SPA $\Delta$ <i>ubiG</i>		$\Delta$ <i>ubiG</i> (cured with pCP20) + P1/ <i>ubiH</i> -SPA::Kan	this work
MG1655 <i>ubiH</i> -SPA $\Delta$ <i>ubiI</i>		$\Delta$ <i>ubiI</i> (cured with pCP20) + P1/ <i>ubiH</i> -SPA::Kan	this work
MG1655 <i>ubiF</i> -SPA $\Delta$ <i>ubiI</i>		$\Delta$ <i>ubiI</i> (cured with pCP20) + P1/ <i>ubiF</i> -SPA::Kan	this work
MG1655 <i>ubiG</i> -SPA $\Delta$ <i>ubiI</i>		$\Delta$ <i>ubiI</i> (cured with pCP20) + P1/ <i>ubiG</i> -SPA::Kan	this work
MG1655 <i>ubiI</i> -SPA $\Delta$ <i>ubiK</i>		$\Delta$ <i>ubiK</i> (cured with pCP20) + P1/ <i>ubiI</i> -SPA::Kan	this work
MG1655 <i>ubiJ</i> -SPA $\Delta$ <i>ubiK</i>		$\Delta$ <i>ubiK</i> (cured with pCP20) + P1/ <i>ubiJ</i> -SPA::Kan	this work
MG1655 <i>ubiE</i> -SPA $\Delta$ <i>ubiC</i>		$\Delta$ <i>ubiC</i> (cured with pCP20) + P1/ <i>ubiE</i> -SPA::Kan	this work

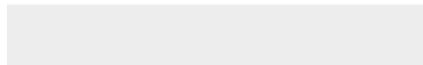
**Table S6** related to figure 1: Oligonucleotides used in this study

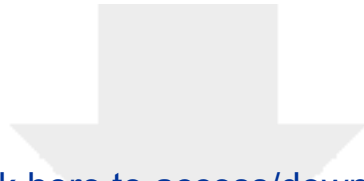
name	category	sequence
	<b>SPA-tag</b>	
5-ubiD-SPA		attgagccatctgggatgaactggctatttttaacaacggtaaaagcgccTCCATGGAAAAGAGAAG
3-ubiD-SPA		catgcgctttctgtcggatcgataaatagggcaaaacaacgcatcaCATATGAATATCCTCCTTAG
5-ubiE-SPA		cgactactacaatctgacggcaggggtgtggcgctgcatcgtggtataagttcTCCATGGAAAAGAGAAG
3-ubiE-SPA		cctgccgtcactaaaggtttaaaaggcatttccggctcctgtcaCATATGAATATCCTCCTTAG
5-ubil-SPA		atccgccaggcaatgggattaacgatttgctgaatggctgctTCCATGGAAAAGAGAAG
3-ubil-SPA		gatgaaagtgtgatgggtatcaataaacaacagaggagaatttttaCATATGAATATCCTCCTTAG
5-ubij-SPA		gctgttgatgccctgaccaaacggctggaaaaactggaggctaaaTCCATGGAAAAGAGAAG
5-ubij-SPA		agtgcgaatgatgaaatataggcgctacttcaactggctcatttagcctccttaCATATGAATATCCTCCTTAG
5-ubiK-SPA		gagatcaaaaagcagccagatccagagactctccccaacgctTCCATGGAAAAGAGAAG
3-ubiK-SPA		ttagaggattaattcataatttacagtgcattaaagacgaatgttaCATATGAATATCCTCCTTAG
5-atpB SPA		ttcatggttctgacgctctatctgtcgtggcgtctgaagaacatTCCATGGAAAAGAGAAG
3-atpB SPA		tttccatgacagtctccagttgtttcagttaaaacgtagtagtgggtaaaCATATGAATATCCTCCTTAG
	<b>bacterial two-hybrid</b>	
5xbal-ubiA		GACTCTAGAGATGgagtggagtctgacgc
3xbal-ubiA		TCCGTCTAGAtcagaaatgccagtaactcattgcc
5xbal-ubiB		TCGACTCTAGAGATGACGCCAGGTGAAGTACGGCGC
3xbal-ubiB		CTTCCGTCTAGATCAGCGTGTTTTGCGCCAACCGACAAACCAGG
5xbal-ubiC		TCGACTCTAGAGATGtcacaccccggttaacgcaactg
3xbal-ubiC		CTTCCGTCTAGATTAgtaacaacggtgacgcc
5xbal-ubiD		TCGACTCTAGAGatggagcgcctgaaatataacgatttacgc
3xbal-ubiD		CTTCCGTCTAGAtcagcgcgtttaccgttgtaaaaatagccagttcatcc
5xbal-ubiE		TCGACTCTAGAGATGGTGGATAAGTCACAAGAAACGCAC
3xbal-ubiE		CTTCCGTCTAGATCAGAACTTATAACCACGATGCAGCGC
5xbal-ubiF		TCGACTCTAGAGATGACAAATCAACCAACGGAAATTGC
3xbal-ubiF		CTTCCGTCTAGACTACAACCCTAACGCATATTTTCAGC
5xbal-ubiG		TCGACTCTAGAGATGaatgccgaaaatcgccgtaaacataacgtagac
3xbal-ubiG		CTTCCGTCTAGAtcactattctgctgtgagcagcatatagttcac
5xbal-ubiH		GACTCTAGAGATGagcgtaatcatcgtcgg
3xbal-ubiH		TCCGTCTAGAtcaacgcgccaccaaccgagggtgcg
5xbal-ubil		TCGACTCTAGAGATGCAAAGTGTGATGTAGCC
3xbal-ubil		CTTCCGTCTAGATTAAACGCAGCCATTAGGCAAATC
5xbal-ubij		TCGACTCTAGAGATGCCTTTTAAACCTTTAGTGACG
3xbal-ubij		CTTCCGTCTAGATCATTAGCCTCCAGTTTTTCCAGCCG
5xbal-ubik		TCGACTCTAGAGATGATTGACCCGAAAAAAATTGAG
3xbal-ubik		CTTCCGTCTAGATTACAGCGTTGGGGGGAGAG
5xbal-ubiX		TCGACTCTAGAGATGAAACGACTCATTGTAGGCATCAG
3xbal-ubiX		CTTCCGTCTAGATTATGCGCCCTGCCAGCG



[Click here to access/download](#)

**Supplemental Videos and Spreadsheets**  
**Table S1.xlsx**





[Click here to access/download](#)

**Supplemental Videos and Spreadsheets**  
**Table S2.xlsx**

